Plasticity in the Reproductive Strategies of the Southern Ocean Ommastrephid Squid *Todarodes filippovae* – a Morphometric, Lipid and Fatty Acid Approach

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Submitted in fulfillment of the requirements of the degree of
Master of Science

University of Tasmania (October, 2005)

DECLARATION:

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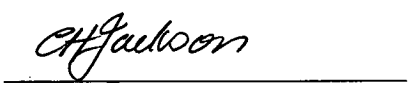

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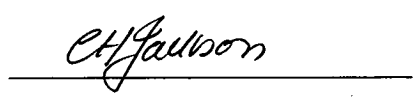

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ABSTRACT

Squid are important trophic links and integral components of the marine ecosystem in the Southern Ocean. Moreover, their life histories have been shown to be extremely plastic thus enabling them to respond quickly to changes in the environment. As a result squid are able to survive and reproduce without the threat of population collapse or annihilation. An examination of female reproductive plasticity in the poorly understood ommastrephid species, *Todarodes filippovae*, revealed both annual variation in condition and gonad investment. This was most likely due to differing oceanographic conditions between the years and seasons investigated. Furthermore, higher gonad investment in females caught in the winter was possibly due to the fact that spawning coincided with the forthcoming spring bloom. It is suggested that *T. filippovae* may spawn in a number of slope and seamount regions across its distributional range. On the terminal versus multiple spawning continuum, results from this study suggested that *T. filippovae* was towards the multiple spawning end. However, some degree of plasticity was associated with this spawning strategy, with one autumn sample of mature females exhibiting a shift away from the multiple spawning end of the continuum.

Female individuals of *T. filippovae* seemed to fuel maturation through the direct acquisition of food. Although there was evidence of a small tradeoff between digestive gland lipid and mantle lipid with maturation, there was no evidence that lipid in the digestive gland was being mobilised as a significant source of energy for oogenesis. Rather it appears that lipid in the digestive gland was excess to maternal nutritional requirements for egg development. The major differences in the relative levels and amounts of lipid classes and fatty acids between the immature and mature ovaries of females signified the important nutritional role of lipid for the young of this species. The role of lipid in the ovary and oviduct was structural rather that as an energy reserve, with maternal provisioning of DHA and EPA most likely critical for the growth and survival of the embryo and rhynchoteuthion. The fatty acid profile of the mature ovary and oviduct varied little temporally suggesting that the quality of the egg was conserved. However, seasonal differences in lipid content and lipid class may have reflected the reproductive plasticity of female individuals of *T. filippovae* in response to environmental conditions at time of capture.

ACKNOWLEDGMENTS:

I wish to express my sincere thanks and appreciation to the many people who assisted and supported me throughout this course of study. Firstly, I would like to thank my supportive team of supervisors to whom I am most grateful. I would like to express my sincere thanks to Assoc Prof Andrew McMinn for his overall support of the project. A big thank you must go to Dr Simon Wotherspoon for his untiring support, assistance and readiness to freely give of his time. Also his willingness to allow me the scope to follow my own ideas was much appreciated. His statistical expertise was invaluable and provided a wonderful learning experience. Thanks also must go to Dr Patti Virtue for always being there and assuring me I could do it. I am very appreciative of her expressed enthusiasm and willingness to help throughout the entire project, including her much appreciated assistance and 'hands on' training in the lab. I am also most grateful to Dr Peter Nichols for facilitating access to state-of-the art facilities at CSIRO for the lipid analysis. His ever-willing assistance in the lab and training in analytical techniques, as well as prompt feedback was much appreciated. Finally, I am grateful for all the constructive feedback received throughout the project from my supervisors.

The Herman Slade Foundation and the Australian Research Council funded this project. Special thanks must go to the captain (Brian Cooksley) and crew of the Adriatic Pearl who were extremely helpful and always went out of their way to collect squid specimens. This project would not have been possible without their assistance. I am grateful to Tertia Macarthur and Heidi Pethybridge who assisted in dissecting some of the squid. Also thank you to Wendy Fox and Talisa Jackson who made excellent data scribes. I am also extremely grateful to Heidi Pethybridge for assistance in the lab, interesting discussions and a willingness to share data. For the ageing of specimens I am grateful to Dr Lianos Triantafillos and Dr George Jackson. Sincere thanks to Dr Alistair Hobday who provided the sea surface temperature and sea surface colour data used in this thesis. Thanks also to Matthew Miller and Danny Holdsworth who managed the CSIRO GC and GC-MS facilities. Dr Julia Jabour also gave much appreciated assistance at the "last hour" in formatting the thesis for printing.

I am indebted to my friends who at times assisted in entertaining my children (much to the delight of the children). I would like to express my sincere gratitude to Wendy and Trevor. A special thanks to Talisa, Adam and Levi who gave their support and understanding throughout the project which enabled this thesis to come to fruition. I appreciated their humour, interesting discussions and potential 'stories' for my thesis. Last but not least I would like to thank my husband George, whose contagious enthusiasm introduced me to the world of cephalopods. I am most grateful for the many interesting discussions, and for his patience when searching for papers in his library to only find 'empty' files. I would like to dedicate this thesis to George for all his persistent support throughout the project that enabled me to keep going even when the going was hard.

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CHAPTER ONE
GENERAL INTRODUCTION

Squid are invertebrates inhabiting a diverse array of ecological niches as evidenced by their ubiquitous presence from the poles to the equator, including neritic to open ocean waters, and from abyssal depths to the epipelagic (Portner & Zielinski, 1998; Forsythe, 2004). Despite their molluscan constraints, squid are also successful competitors in environments dominated by their vertebrate teleost counterparts (O'Dor & Webber, 1986). Within the trophic web of these habitats, squid perform a key role as both predators and prey (for reviews see Rodhouse & Nigmatullin, 1996; Croxall & Prince, 1996; Clarke, 1996; Klages, 1996; Smale, 1996). Historical data has indicated a worldwide depletion of up to 90% of large predatory fishes (Myers & Worm, 2003). Similarly, either prior or simultaneous to the decline in predatory fish, there has also been a reduction in other predators of cephalopods such as toothed whales (Caddy & Rodhouse, 1998). This major change in the structure of marine ecosystems has resulted in a decline in the mean trophic level of species being fished (Pauly et al., 1998). This shift in landings represents an overall transition from long-lived fish species that occupy a higher trophic level, to short-lived invertebrates and fish at a lower trophic level (Pauly et al., 1998). A decrease in predatory pressure and increased food resources may partially account for the rise in cephalopod landings observed in recent decades (Boyle & Boletsky, 1996; Caddy & Rodhouse, 1998; Rodhouse et al., 2001).

The possible role of squid as signals of worldwide oceanic changes in fishery exploitation (Rodhouse, 2001) highlights their ability to adapt rapidly to not only anthropogenic change but also to natural changes in the environment. Their teleost competitors typically have slow growth with an extended asymptotic growth phase, multi-year spawning and lifespans measured in years, decades or even over a century (Fenton et al., 1991). In contrast, squid life histories are characterised by annual or sub-annual lifespans, rapid indeterminate growth (Jackson, 2004), little overlap in generations (Boyle & Boletsky, 1996), efficient feeding/digestion and a rapid protein-based metabolism (O'Dor & Webber, 1986). It is these distinctive life histories exhibited by squid that enable them to maximize their survival in competitive and changeable environments (McGrath-Steer, 2004). As a result there is

extreme measurable plasticity in both growth and reproductive tactics (Villanueva, 1992; Hatfield & Cadrin, 2002; Jackson et al., 2003; McGrath-Steer & Jackson, 2004; Pecl et al., 2004). There are constraints on the inherent plasticity of squid due predominantly to their short lifespans and dependence on short-term environmental variations in abiotic and biotic conditions. Extreme and acute changes in the environment during the 1997-88 El Niño resulted in the collapse of the *Loligo opalescens* fishery in California (Butler et al., 1999). Although not always successful, as evidenced for *L. opalescens* and as documented for *Illex illecebrosus* (O'Dor & Dawe, 1998), the best buffer against population collapse is risk spreading that may occur in several dimensions including time, space and phenotype (O'Dor, 1998).

At any particular age an organism is faced with the decision to reproduce or not (Bell, 1980). Individuals that are able to spread their reproductive effort over seasons or years may benefit from increased fecundity with age or from the confidence of higher offspring survival at some point in time when conditions are optimal (Kerkendall & Stenseth, 1985). Due to their short life spans, squid lack the reproductive reserves of multiple year classes afforded to teleost fish (O'Dor, 1998), and it is evident that there is a great deal of flexibility in the reproductive strategies adopted by squid (Boyle et al., 1995; Pecl, 2001; Rocha et al. 2001) Historically, squid have been termed semelparous spawners i.e., they were thought to spawn once and die. A pivotal paper by Harman et al. (1989) documented that multiple spawning was also a reproductive strategy employed by squid as verified in the ommastrephid *Stenoteuthis oualaniensis*. Mangold et al. (1993) further reiterated that squid exhibit both semelparity and non-seasonal interoparity, and that a species is likely to display a strategy somewhere along a continuum between these two reproductive modes (e.g., Collins et al., 1995; Melo & Sauer, 1999; Pecl, 2001; McGrath & Jackson, 2002).

Given that population structure is largely determined by phenotypic flexibility in response to changeable environments (Boyle & Boletsky, 1996), it is doubtful whether a squid species will consistently display one reproductive trait (McGrath-Steer, 2004). Since the long-term goal of any species must be for the survival of the subsequent generation, phenotypic plasticity would be a hedge against all plausible

environmental conditions (O'Dor, 1998). Under certain conditions abiotic and biotic factors may force a reproductive trade-off, whereby one process is benefited at the expense of another (Begon et al., 1986). Any trade-off is likely to result in a lower cost-benefit ratio in relation to growth and survival for both the female and progeny (Forsman, 2001).

The inherent energy reserves available for partitioning between growth and reproduction may largely be a function of timing, that is, when energy reserves are available for gametogenesis (Moltschaniwskyj & Semmens, 2000). Since maturation in squid is predominantly determined by size rather than age (Jackson et al., 1997), any impact due to environmental factors such as temperature or food on the growth process, (i.e. the size, age or condition of the spawning population) will also impact on the level of energy apportioned to the reproductive process (Heyer et al., 2001; Pecl et al., 2004). It is probable that maternal effects resulting from environmental variation will impact heavily on the subsequent population since female energetic investment is critical for proper development and survival of hatchlings (Heyer et al., 2001). In the cephalopod literature there are examples of both field and laboratory studies which have documented reproductive tradeoffs resulting from maternal responses to environmental variation. Jackson (1993) found that slower growing cool-season individuals of the sepiod *Idiosepius pygmaeus* had an extended lifespan and relatively larger gonads, and an inferred greater reproductive output than those individuals growing through the warmer season. Experimental evidence showed that maturation and subsequent egg laying in *Sepioteuthis lessoniana* was accelerated under higher temperatures due to increased growth rate (Forsythe et al., 2001). Low maternal ration apportioned to *I. pygmaeus* resulted in smaller clutches of eggs being laid (Lewis & Choat, 1993) while the sepiolid *Euprymna tasmanica* traded fecundity and egg size for egg quality (Steer et al., 2004).

As has been suggested for teleosts, the reproductive success of squid may be related to their spawning habitat. Demersal spawners would be expected to have more control over their spawning habitat and thus reduce the variation experienced by the hatchlings. This necessitates fewer but larger eggs. Therefore reproductive success is likely to be highly individualistic, with success increasing with egg size (Duarte & Alcaraz, 1989). In contrast, teleost pelagic spawners may increase reproductive

success by the sheer number of spawned eggs. By maximizing the number of eggs produced, it is likely that some hatchlings will encounter suitable feeding areas in a patchy environment. The reduced time between spawning and hatching resulting from a shorter incubation time required for smaller eggs ensures greater certainty regarding the hatching location (Duarte & Alcaraz, 1989). This contrast between demersal and pelagic teleost spawners bears similarity to that observed between loliginid squids which lay egg mops consisting of fewer larger eggs, to that observed for ommastrephid squid species which spawn numerous (> 1 million for *Illex argentinus*) smaller eggs (Laptikhovsky & Nigmatullin, 1993).

1.1 Research rational and structure

The rationale for this study was to assess and explore the nature of reproductive tactics and its intrinsic plasticity in the poorly understood squid *Todarodes filippovae*. *Todarodes filippovae* is an ommastrephid species approaching 400-500mm mantle length (ML)(Dunning, 1993), about mid-size between the minute *Todarodes pacificus pusullus* (15g) (Dunning, 1988a) and the large *Dosidigas gigas* reaching 120kg (Wormuth, 1998). Although ommastrephid species occur in most latitudes from the tropics to almost the poles (Roper et al., 1984), *T. filippovae* is a circumpolar squid spanning the sub-Antarctic and the subtropical zones of the Southern Ocean (Rodhouse, 1998). Many ommastrephid species are associated with current systems and as such undertake both large (up to 2000 km for *Illex illecebrosus,* O'Dor et al., 1997) and small scale migrations, with the scale of the migration most likely correlated with the strength of the associated current (Hatanaka et al., 1985). Like all ommastrephids, *T. filippovae* has a spawning mode consisting of gelatinous egg masses or 'balloons' (O'Dor & Balch, 1985) that float freely in the water column and can be transported in ocean currents (O'Dor et al., 1997). The reproductive cycle of ommastrephids is environmentally driven. For example, *I. illecebrosus* egg masses are spawned off Florida, and egg masses and young are transported north in the Gulf Stream to feeding grounds off North-eastern America. The cycle is completed when the mature population returns to the spawning grounds off Florida to ensure embryonic development and hatchling success in warmer waters (O'Dor & Dawe, 1998). Given the close association between the life cycle of *I. illecebrosus* and oceanographic features (Dawe et al., 2000), and for ommastrephids generally, it is likely that environmental variation will be reflected in phenotypic

plasticity. In the southeast Australian region *T. filippovae* is the most abundant ommastrephid in continental slope waters (Dunning, 1988b). Therefore, it is of considerable importance to understand the reproductive biology of this species, which must play a significant role in the ecology of this environment.

There are two known species of *Todarodes* in Southern Ocean waters, *T. filippovae* and *T. angolensis*. Although these two species are both captured in waters off Tasmania, *T. filippovae* is much more abundant. Both species are easily distinguished based on the size of the largest median manus tentacular sucker, as well as the number of teeth on the median manus sucker ring (Roeleveld, 1989; Dunning & Wormuth, 1998). On freshly defrosted specimens *T. angolensis* also has a lighter red skin pigmentation compared to the deeper red pigmentation of *T. filippovae*.

This thesis encompassed two major research areas in assessing life history parameters in relation to the reproduction of *T. filippovae*. The overall structure of the research is in the form of two data chapters (Chapters 2 and 3) written in a manuscript format suitable for journal submission. The overall aims of each chapter are as follows:

1.2 Chapter two:

All research for this and the subsequent chapter was carried out on female specimens of *T. filippovae*. This was due to very few male specimens being trawled. Very little is known of the reproductive biology of *T. filippovae*, so an analysis using morphometric data on females spanning three years and three seasons was conducted to elucidate temporal responses in condition and gonad investment. To determine if these responses may be environmentally driven, they were explored in terms of lagged sea surface temperature (SST) and sea surface colour (SSC). Furthermore, the overall reproductive strategy (terminal versus multiple spawning) was determined via morphometric analysis and examined temporally to determine if there was any degree of phenotypic plasticity within that strategy.

1.3 Chapter three:

This chapter extends the findings of chapter 2 by examining the role of lipid in the reproductive process of females. Lipids have repeatedly been advocated as a fuelling mechanism for maturation via storage in the digestive gland. To determine if lipid was a source of energy for reproduction in this species, the inter-relationship between lipid content in the mantle, digestive gland and ovary was analysed in concert with maturation. Although squid have a protein-based metabolism, it was of interest to explore the exact structure and function of lipid in the gonad given the requirement of particular lipids and fatty acids in all cells, which may infer specific requirements for the rhynchoteuthion. Moreover, since the females caught in winter examined in chapter 2 had greater gonad investment than summer and autumn caught females, the role of maternal provisioning in relation to lipid in the mature ovary and oviduct was examined temporally.

CHAPTER TWO

TEMPORAL CHANGES IN THE CONDITION AND REPRODUCTIVE INVESTMENT OF *TODARODES FILIPPOVAE* - A PLASTIC RESPONSE TO THE ENVIRONMENT

2.1 Abstract

Annual and seasonal trends in reproductive parameters were examined for 378 female individuals of *Todarodes filippovae* caught in waters off southern and eastern Tasmania, Australia. Samples were collected from the commercial deep-sea fishing industry during summer, autumn and winter over a three year period from 2002-2004. Both condition and gonad investment of mature females was significantly poorer in 2002 than in the subsequent two years. Gonad investment was also dependent on season with the smaller winter females investing greater in gonads than their summer or autumn counterparts. There was an observed decreasing trend in sea surface temperature (SST) over the three years. Sea surface colour (SSC) during the study period was dominated by a large peak in chlorophyll *a* in late summer/autumn of 2003. While correlation analysis suggested that gonad investment correlated with SSC at time of capture, there was a two-month lag between SSC and condition. No signal was detected between condition or gonad investment and SST. *Todarodes filippovae* exhibited a reproductive strategy indicative of multiple spawning. The lack of any negative correlation between ML-somatic residuals and ML-gonad residuals suggested that at the whole animal level there was no evidence of energy being diverted away from somatic growth during maturation. A concomitant increase in condition and gonad investment was documented for 2003 and 2004 autumn females ($p<0.05$). Further evidence for multiple spawning was a lack of correlation between ML and oviduct fullness for any season in any year with the exception of autumn 2003. The positive correlation between ML and oviduct fullness for that season ($r=0.52$, $p<0.05$), coupled with some evidence of ovary depletion, suggests a shift towards the terminal end of the spawning continuum. However, there was very little evidence that mature females in other seasons were depleting their ovaries of oocytes as the eggs were transferred to the oviducts. Mature females are likely to fuel the maturation process through the direct acquisition of food. The reproductive mode and marked plasticity observed in the reproductive life history of *T. filippovae* were likely to be biological responses to patchy and changeable environmental conditions.

2.2 Introduction

Squid populations are characteristically unstable (Rodhouse, 2001) and extremely plastic in their response to varying environmental conditions (e.g. Villanueva, 1992; Hatfield, 2000; Ichii et al., 2004; McGrath-Steer, 2004; Pecl et al., 2004). A short lifespan, up to a year for most species (see reviews Arkhipkin, 2004; Jackson, 2004) coupled with a cephalopod lifestyle defined by rapid, indeterminate growth, i.e. 'life in the fast lane' (Jackson & O'Dor, 2001) is a primary contributing factor to this plasticity (Boyle & Boletsky, 1996). Squid are also opportunistic thus enabling them to rapidly exploit favourable conditions (Rodhouse, 2001). The 'fast life style' of squid also makes them extremely responsive to environmental change and ideal marine models for examining a variety of biological parameters.

Since cephalopod populations in general are highly influenced by the interaction between physical and biological factors over extremely short time spans (Boyle & Boletsky, 1996) it is imperative to not only have an intimate knowledge of their life cycle, but an understanding of the oceanographic conditions of their habitat and its inherent variability (Anderson & Rodhouse, 2001). Due to the rapid growth and turnover of cephalopod populations (Boyle & Boletsky, 1996), it is unlikely that successive cohorts will experience similar conditions, resulting in a high degree of phenotypic (McGrath- Steer, 2004) and recruitment variability (Sakurai et al., 2000; Waluda et al., 2001). Furthermore, since growth and reproduction are inextricably linked (Mangold et al., 1993), it is probable that plasticity in growth (intra-specific variation) will lead to highly individualistic reproductive tactics. Consequently, population responses to environmental fluctuations in prey abundance or thermal conditions, for example, may be manifested by plasticity in maternal condition and reproductive strategies (Brown & Murphy, 2004; McGrath- Steer, 2004; McGrath-Steer & Jackson, 2004), thus demonstrating mechanistic links between environmental conditions and life history patterns (Pecl et al, 2004).

The early life stages of cephalopods are extremely responsive to temperature (Forsythe, 1993) or temperature-mediated parameters, such as food availability (Robin & Dennis, 1999). Furthermore, cephalopods have an extended juvenile phase (Jackson, 2004) and population survival and success may be determined by the degree of plasticity in an individual's reproductive strategy. The type of spawning

strategy used largely determines maturation and reproductive allocation in squid. For example, cephalopod species with large reproductive investments may have a terminal reproductive strategy (e.g. Jackson et al., 2004), whereas species making smaller investments may lay a number of smaller batches of eggs over an extended period of time (e.g. Melo & Sauer, 1999; McGrath & Jackson, 2002). Unlike many fish that store energy for maturation, future reproductive events or over-wintering (Craig et al., 2000; Lambert & Dutil, 2000; Cubillos et al., 2001; Abitia-Cardenas et al., 2002), cephalopods have a limited ability to store energy and rideout lean times (O'Dor & Webber, 1986). However, some squid species cease to feed and derive their energy for oocyte maturation and spawning by sequestering energy from the soma (Fields, 1965; Jackson & Mladenov, 1994). In contrast, other species continue to feed but preferentially divert energy from growth of the soma to reproduction (Hatfield & Rodhouse, 1992; Collins et al., 1995), while others may continue to feed and grow (Harman et al., 1989). As a consequence, food is likely to be a critical driving force in the variability of female condition and reproductive investment due to short-term changes in the environment (Pecl et al., 2004).

Although the intrinsic plasticity of many squid life histories is becoming well established, there is a paucity of information documenting this flexibility in many Southern Ocean species. *Todarodes filippovae* Adam 1975, sometimes referred to as the Southern Ocean arrow squid, is a large, sexually dimorphic ommastrephid species with a circumpolar distribution, found in the Southern Ocean south of 35°S. It inhabits primarily temperate oceanic and slope waters (Dunning & Brandt, 1985; Dunning, 1998; Wadley & Dunning, 1998) and is commonly associated with the Subtropical Convergence Zone (Roper et al., 1984). *Todarodes filippovae* is reported to be the most common ommastrephid in slope waters along the southeastern Australian coast as well as in the sub-tropical convergence zone of the Tasman Sea (Dunning, 1988b). Many ommastrephids undertake large migrations in association with high velocity current systems (O'Dor, 1992), which assist in the transport of planktonic eggs and paralarval stages (Rodhouse, 1998). However, there is no evidence thus far to suggest that *T. filippovae* undertakes such migrations off Eastern Australia (Dunning, 1993). *Todarodes filippovae* is an important link in the trophic web and is recorded as prey for sperm whales (Dunning, 1988b), southern elephant seals (Slip, 1995; Burton & van den Hoff, 2002), black-browed albatross, yellow

nosed albatross and grey-headed albatross (Cherel & Klages, 1997). Furthermore, based on stomach content analysis in conjunction with lipid and fatty acid analysis, a primarily piscivorous diet dominated by myctophids was documented for *T. filippovae* caught in Tasmanian waters. Both squid and crustaceans were considered incidental rather than dominant prey items (Pethybridge, 2004).

In the central Tasman Sea and off the east Australian coast *T. filippovae* have been captured by jigs, driftnets and in midwater trawls. In trawl catches females predominated (up to 7 times more abundant than males), however, sex ratios were similar in jig catches (Dunning 1993). Data supports a one-year life cycle for this species with large females typically ranging in size from 400-500 mm mantle length in the central Tasman Sea. However, only a few mature females were caught. In contrast, in adjacent eastern Australian continental slope waters, mature specimens of both sexes were captured during summer months. Furthermore, mature females ranging in size from 410-474 mm mantle length have been trawled off eastern Tasmania in late summer and early winter (Dunning 1993). Protracted spawning, typical of many ommastrephids is highly likely in this species (Rodhouse, 1998), even though discrete spawning periods may occur in the austral summer and midwinter (Dunning, 1988b).

The overall rationale for this study was to investigate seasonal and annual variation in the female reproductive strategy of the important but poorly understood Southern Ocean squid species *T. filippovae*. More specifically, the aims were to examine temporal changes in condition and reproductive investment to assess resource allocation and any inherent flexibility in reproductive parameters. In addition, a separate aim was to determine the spawning strategy of *T. filippovae*, whether multiple or single, and if there was any temporal plasticity within that strategy.

2.3 Methods

2.3.1 Collection details

Samples were collected over three seasons for three consecutive years to determine the extent of seasonal and annual reproductive plasticity. The squid were obtained incidentally from the commercial deepwater trawl fishing industry off southern and

eastern Tasmania from a number of trips each season. Squid were frozen upon capture, although 17 squid (4.5%) were kept on ice and dissected fresh. Once defrosted, data collected for each squid included, sex, dorsal mantle length (ML, mm), total body weight (BW, g) and somatic weight (combined mantle + fin weight). Similarly, the weight (g) of all the reproductive organs was recorded, that is, ovary (OV), nidamental gland, oviducal and oviduct weight (weight of eggs accumulated prior to spawning). Each specimen was also assigned a maturity stage (after Lipinski, 1979), based on the size and colour of the reproductive organs. A reproductive-somatic index (after Pecl, 2001) was calculated for each squid as:

$$RSI = TRW/(BW - TRW) *100$$

where TRW is the total reproductive weight, defined as the sum of the combined weights of the ovary, nidamental gland, oviducal gland and the oviduct.

Each female was also examined for mating as indicated by the presence of spermatophores around the buccal membrane or stored sperm in the seminal receptacles. The stomach of each specimen was assigned a fullness stage ranging from one to five, with one indicating very little food in the stomach while five indicated a stomach that was very full and distended (after Jackson et al., 1998).

All statoliths were removed, rinsed with water and stored dry at room temperature. For increment counting, statoliths were mounted in the thermoplastic cement Crystal Bond and ground and polished on the anterior and posterior planes using lapping film (after Jackson et al., 2003). Increment resolution near the edge was enhanced for many of the statoliths by tilting and grinding the posterior (convex) surface on an angle, running from the nucleus to the edge. Total increment number was taken from two consecutive counts that differed less than 10% of the mean. Counts were taken directly using a compound microscope or viewing an image on a PC computer screen via a digital camera. Total increment count was used as putative individual age.

2.3.2 Temporal changes in condition and gonad investment

Two statistical methods were applied to the data to assess annual and seasonal differences in condition and gonad investment. Firstly, a residual analysis based on

type II geometric regressions that adjusted for the size of the animal was used (after Green, 2001; McGrath-Steer, 2004). Secondly, a multivariate approach using canonical discriminant analysis was used to predict group membership of year and season separately based on a number of log-transformed morphometric measures. These included ML, somatic wt, OV weight, nidamental gland weight, oviducal gland weight and oviduct weight. Discriminant analysis was applied to determine if differences between seasons and years could be attributed to functions that were consistent with the residual analysis.

Two length-weight type II geometric regressions were calculated on log-transformed data. Log mantle length was used as the independent variable while log somatic weight (a combined mantle + fin wt) and log gonad weight were used as dependent variables. This analysis is based on the premise that animals with ML-somatic weight observations placed above that predicted (regression line) have a positive residual and are therefore in relatively better condition than those individuals with ML-somatic weight observations below the predicted, resulting in a negative residual. Similarly, observations above the predicted line for the ML-gonad regression indicate animals with greater gonad investment as opposed to those animals with observations that fall below the line, which are thought to have lower gonad investment. Two-way full factorial analysis of variance (ANOVA) with season and year as the factors of interest was used to determine differences in unstandardised condition residuals and unstandardised gonad investment residuals. When a significant interaction or main effect was obtained in the factorial models, a Tukey's honestly significant post-hoc test was computed to determine where the significant group differences were occurring. For these and all subsequent analyses, unless otherwise stated, mature females included both stage 4 and 5 females. Stage 4 females were considered functionally mature, even though there were no mature oocytes in the oviducts.

A third type II geometric regression was used to determine the relationship between age and BW, which were also both log-transformed. Animals with positive residuals from the age-BW regression were considered to be the faster growers in comparison to slower growers that had negative residuals. Correlations between the age-BW residuals and the ML-gonad residuals were used to determine if faster growers also

had greater gonad investment. Likewise, age-BW residuals were correlated with ML-somatic residuals to ascertain if those with a faster lifetime growth rate were in better condition.

2.3.3 Sea surface temperature and sea surface colour

Sea surface colour (SSC) was used as a proxy for chlorophyll *a* concentrations (after Jackson et al., 2003) to explore the relationship between productivity in the study region with condition and gonad investment. Eight-day chlorophyll *a* composites were obtained from SeaWifs (Sea-viewing Wide Field-of-view Sensor) from January 2001 to April 2004. From May 2004 to December 2004 when no SeaWifs data was available, MODIS daily images were used. Similarly, parallel 1-3 day SST time series were obtained (to August 2004) from the NOAA-CIRES Climate Diagnostic Center (available at www.cdc.noaa.gov/). Although the extent of movement patterns in relation to spawning activity are unknown for this species, it was assumed for the purposes of this study, that SST and SSC in the area of sampling would be representative of conditions experienced by mature squid prior to capture. The SSC data for mature summer and autumn squid trawled off southern Tasmania were derived based on a box encompassing much of the sampling area (44 - 46°S, 145-152°E) while SST was obtained for a 1° box (45°S, 148°E). For mature winter caught squid a 1° box off eastern Tasmania was used (41°S, 149°E) to estimate both SSC and SST. These SSC and SST data were then averaged to provide a monthly time series that corresponded to the study period.

Both ML-somatic weight residuals (condition) and ML-gonad weight residuals (gonad investment) were cross-correlated with time lagged SSC and SST to investigate temporal associations between condition and gonad investment and prevailing environmental conditions. Since residuals were grouped according to austral seasons, correlation analysis was based on lagged 3 monthly moving averages of SST and SSC. Each three-month period (season) was identified by the first month of that season. Standard errors for the correlations were estimated by drawing 1000 bootstrap samples within seasons and years.

2.3.4 Energetic trade-off

To determine if *Todarodes filippovae* employ a reproductive-somatic trade-off between the growth of the soma and the growth of the reproductive structures during maturation, type II geometric regression equations on ML-somatic weight and ML-gonad weight were produced for each year and season combination. Females from all maturity stages were used in these regression equations. Pearson r correlations (2-tailed) were performed on the unstandardised residuals from the ML-somatic and ML-gonad regressions of the same sample (after McGrath-Steer, 2004).

2.3.5 Spawning strategy

Oviduct fullness was calculated using all mature (stage 5) females to determine if eggs in the oviduct were being stored for release in 1 large batch or in a number of smaller batches. To predict potential maximum oviduct weight, a linear regression of oviduct weight against ML was fitted assuming exponentially distributed errors (Pawitan, 2001), to reflect the fact that the maximum weight will exceed all observed weights. Under these assumptions the maximum likelihood estimate of the line of best fit is the line that is tangential to the upper convex hull of the observations and minimizes the absolute deviations. Because this method assumes all observations fall under the maximum, it is a less conservative technique than that of Harman et al. (1989). Spearman rank correlation was then used to see if there was any relationship between ML and percent oviduct fullness, which was derived by dividing the actual oviduct weight by the predicted, for each stage 5 female. This enabled us to determine if bigger females were retaining a greater volume of eggs (larger batches) before spawning. A two-way full factorial ANOVA with season and year as the factors of interest was used to determine any differences in average oviduct fullness for each sampling period.

An ovary to oviduct ratio was calculated to determine if the ovary became depleted as eggs moved into the oviduct. Partial correlations were performed on somatic weight and oviduct weight, controlling for total BW. These correlations were used to establish if females in better condition were producing larger batches of eggs.

2.4 Results

A total of 378 female individuals (including 1 juvenile) were collected during the austral summer, autumn and winter over a 3 year period from 2002 to 2004. Trawl depths ranged between 650-1300 m. All samples were a combination of squid from multiple tows over a number of trawling trips. The summer and autumn samples in each year were obtained in waters around southern Tasmania predominantly in the vicinity of Maatsuyker Island, Pedra Branca and the Cascade Plateau (145-152°E, 44-46°S). There was no winter sample available in 2003. The winter sample of 2002 was obtained from waters off the east coast of Tasmania near St Helens (149°E, 41°S) and Bicheno (4 specimens). The 2004 winter sample included females caught in waters off southern Tasmania (as per summer and autumn samples) and from St Helens on the east coast of Tasmania (as for the 2002 winter sample) (Table 2.1). There was no significant difference between the ML, BW and OW of mature females caught from these two locations. Therefore animals from both southern and eastern Tasmania were included in this winter sample. Some tissue weights of four trawl-damaged specimens with missing portions of tissue were estimated by weighing comparable tissue (e.g. the weight of one nidamental gland weight was doubled when one nidamental gland was missing to estimate total nidamental gland weight).

Specimens caught in the autumn attained a maximum size of 540mm ML and 4704g in BW, while summer specimens attained a maximum size of 529mm ML and 3648g BW. Conversely, the largest female captured during the winter seasons was 469mm ML and 2348g BW. Overall, specimens ranged in age from 178 to 364 days (Table 2.1). Correlation analysis revealed that maturity is predominantly a size not age-related process. Growth of the main reproductive organs combined (ovary weight + oviducal gland weight + nidamental gland weight) correlated well with ML and BW across all seasons and years. Generally, age also correlated with the weight of reproductive organs although to a much lesser degree.

Table 2.1: *Todarodes filippovae*. Summary of collection details, including size and age range of all females.

Year	Season	Location	No. sampling trips/no. of days	Total Captured	Mantle Length range (mm)	Total Body Weight range (g)	Total Aged	Age Range (days)
2002	Summer	Southern Tasmania	11	57	221-528	162-3541	56	197-364
	Autumn	Southern Tasmania	8	48	289-540	471-3784	46	214-360
	Winter	Eastern Tasmania	3	26	359-461	951-2268	26	222-330
2003	Summer	Southern Tasmania	6	41	353-518	842-3501	39	240-321
	Autumn	Southern Tasmania	6	63	286-540	487-4704	63	208-337
	Winter	n/a						
2004	Summer	Southern Tasmania	5	37	269-529	379-3648	37	234-345
	Autumn	Southern Tasmania	10	89	230-533	197-3709	87	178-346
	Winter	Eastern/southern Tasmania	3	17	277-469	428-2348	16	232-314

However, no significant correlation was found in the 2004 summer, while the summer of 2003 was only poorly correlated. These two summers exhibited greater variation in the growth of the reproductive organs as indicated by the lower correlations with ML and BW. Females captured in the winter of 2004 also exhibited a poor correlation between the age and weight of maturing reproductive organs (Table 2.2).

Table 2.2: *Todarodes filippovae.* Summary of correlation analyses between combined reproductive weights (ovary + oviducal + nidamental gland) and age, mantle length and total body weight of all females grouped by year and season.

Year/ Season	Age			Mantle Length			Total Body Weight		
	r	p	n	r	p	n	r	p	n
2002 Summer	0.271	0.05	53	0.512	<0.001	54	0.635	<0.001	54
2002 Autumn	0.526	<0.001	46	0.795	<0.001	48	0.923	<0.001	47
2002 Winter	0.597	0.002	24	0.841	<0.001	24	0.874	<0.001	24
2003 Summer	0.017	0.92	39	0.521	0.001	40	0.698	<0.001	40
2003 Autumn	0.574	<0.001	62	0.783	<0.001	62	0.887	<0.001	62
2003 Winter	n/a			n/a			n/a		
2004 Summer	0.616	<0.001	36	0.711	<0.001	36	0.844	<0.001	35
2004 Autumn	0.584	<0.001	87	0.729	<0.001	89	0.869	<0.001	88
2004 Winter	0.497	0.05	16	0.762	<0.001	17	0.883	<0.001	17

2.4.1 Somatic condition and gonad investment

Somatic condition showed evidence of a season by year interaction ($F = 3.18$, $df = 3,158$, $p < 0.05$). However, this must be interpreted with caution due to a missing cell (no 2003 winter sample). When the model was refitted to subsets where there was no missing cell i.e., all summers and autumns over the three years, as well as all summer, autumn and winter samples for 2002 and 2004 with year and season as the factors of interest, the results were consistent with those found for the original model. Condition for all summers and autumns over the three years was also dependent on a

season by year interaction. ($F = 4.125$, $df = 2,129$, $p < 0.05$). However, a comparison between summer, autumn and winter in 2002 and 2004 only showed evidence of a year main effect ($F = 35.709$, $df = 1,106$, $p<0.001$). This suggests that the summer and autumn samples in 2003 were driving the season by year interaction. In the summer of 2002 and 2004, condition was higher than in autumn, however, in 2003 the situation was reversed, with autumn in this year higher than all other samples except for winter 2004. Nevertheless, overall, 2002 specimens were in relatively poorer condition than the two subsequent years (Fig. 2.1).

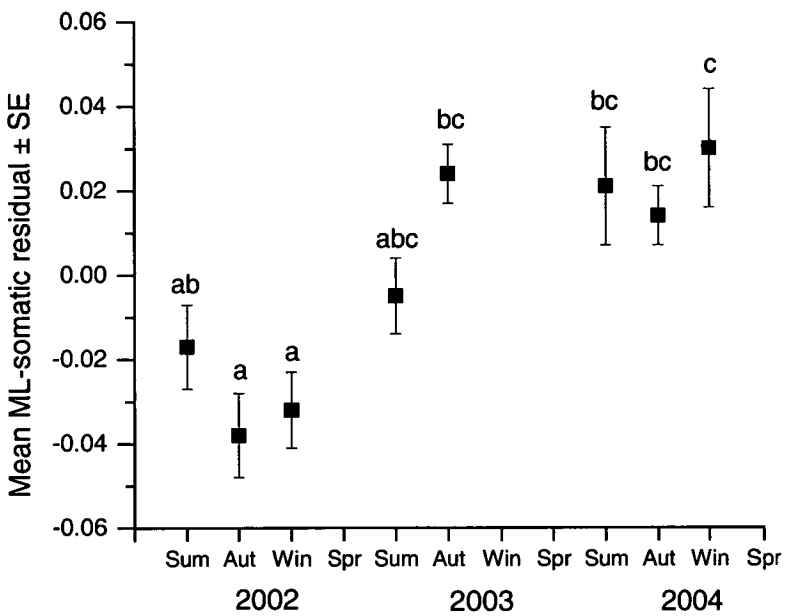

Figure 2.1: *Todarodes filippovae.* Mean residuals from the ML-somatic weight regression for all mature females for each year and season combination. Letters correspond to significant comparisons of means as identified by Tukey's HSD post hoc test, SE=standard error.

Variation in gonad investment was dependent upon season ($F = 41.054$, $df = 2,158$, $p < 0.001$) and year ($F = 12.852$, $df = 2,158$, $p < 0.001$). Mature females had higher gonad investment during winter than in summer and autumn (Fig. 2.2a). Although the omnibus test showed evidence of a year effect, this was not detected by a Tukey's post-hoc test, presumably due to the comparatively lower power of the Tukey's procedure. However, examination of the mean ML-gonad residual showed that 2002 had lower gonad investment than 2003 and 2004 (Fig. 2.2b). Models

applied to subsets of this data, as for somatic condition, showed consistent results to that found for gonad investment among all years and seasons.

There was little evidence to suggest that lifetime growth rate affected condition or gonad investment in any year/season. A faster lifetime growth rate was only found to be significantly associated with a greater gonad investment for mature females caught in the summer of 2002 ($r = 0.644$, $n = 16$, $p<0.01$). Similarly, a faster lifetime growth rate only correlated with an increase in somatic weight relative to ML for 2004 autumn females ($r=0.454$, $n=35$, $p<0.01$)

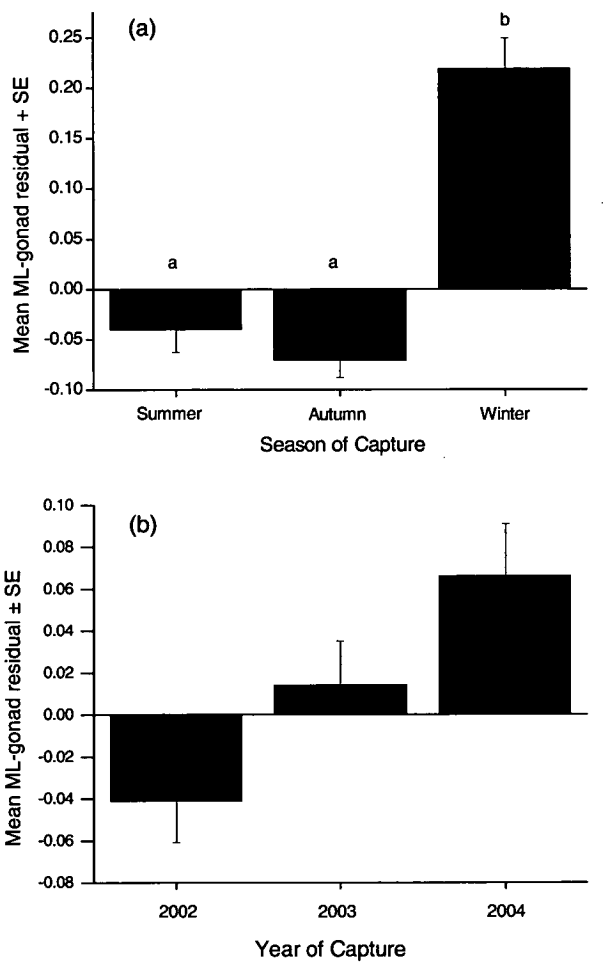

Figure 2.2: *Todarodes filippovae.* Mean residuals from the ML-gonad weight regression for all mature females based on (a) season of capture and (b) year of capture. Letters correspond to significant comparisons of means as identified by Tukey's HSD post hoc test, SE=standard error.

Stepwise linear discriminant analysis on morphometric weights and measurements grouped by year revealed a significant discriminant that accounted for 99.5% of the variance (Wilks' lambda = 0.574, $p < 0.001$). This function was a contrast between increasing somatic weight and decreasing ML. Compared to 2003 and 2004, 2002 had a lighter somatic weight coupled with an increased mantle length (Fig. 2.3a). Discriminant analysis on only 2002 and 2004 with no missing cells gave similar results. Only 54.8% of the original grouped cases were correctly classified into their predicted group membership. However, 79% of cases from 2002 were correctly classified indicating that this year was distinctly different. Conversely, 2003 was classified into all years but predominantly confused with 2004 cases. Similarly, 2004 was mostly confused with those cases belonging to 2003, thus highlighting that 2003 and 2004 were more similar than they were different (Table 2.3).

Stepwise linear discriminant analysis also was used to distinguish any seasonal differences based on morphometic weight and measurements of the squid. There was evidence of a significant discriminant (Wilks' lambda = 0.467, $p < 0.001$) that accounted for 99.9% of the variance. Based on the standardized canonical discriminant function coefficients this function was a contrast between increasing somatic weight and decreasing ovary weight. Winter was grouped according to a lighter somatic weight but increased ovary weight. Conversely, the summer and autumn samples both grouped on increasing somatic weight along with decreasing ovary weight (Figure 2.3b). Discriminant analysis applied to all subsets produced results supporting the full model. Only 58.4% of the original grouped cases were correctly assigned to the predicted groups. However, 93.5% of the winter group was correctly classified. This was in contrast to the summer and autumn groups that were indistinguishable and were unable to be correctly assigned to their predicted group. This suggests that winter samples were distinctly different from summer and autumn based on the somatic weight – gonad weight function (Table 2.4).

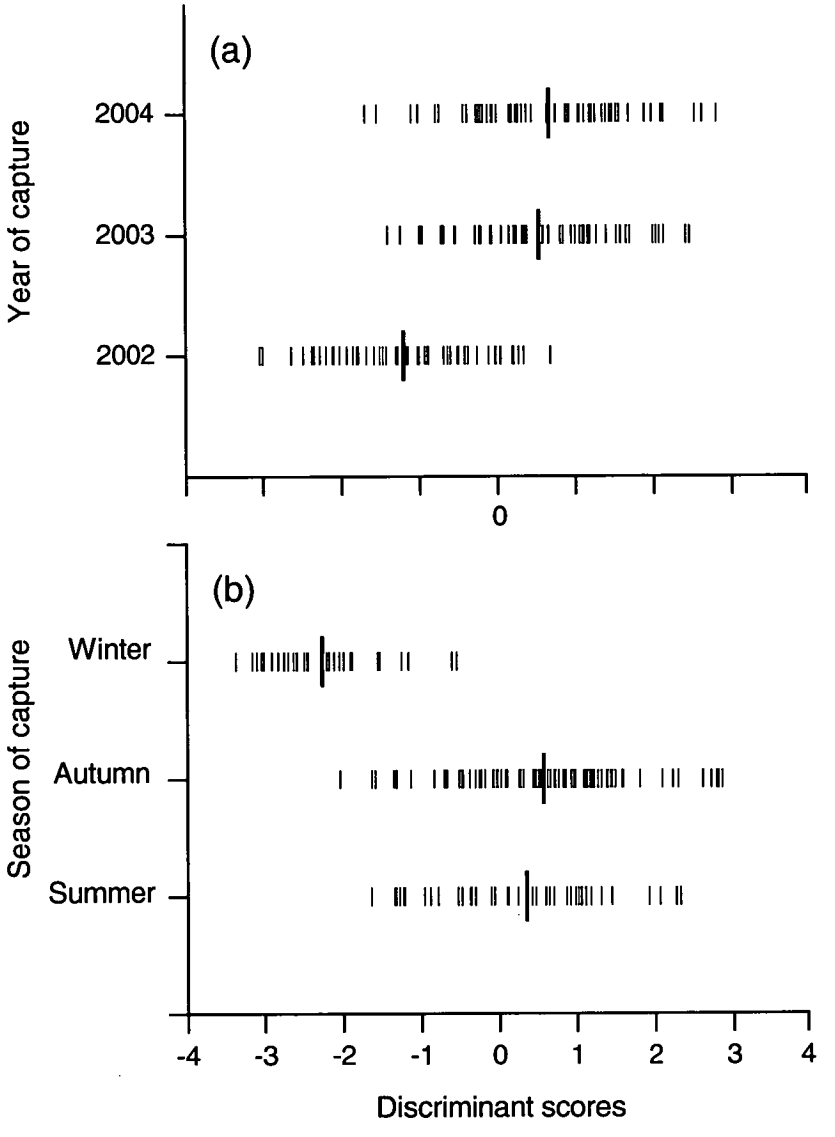

Figure 2.3: *Todarodes filippovae.* Discriminant scores for function 1 from discriminant analyses on selected morphometric weights and measurements of mature females grouped according to (a) year and (b) season. The long dash for each season and year group represents the group centroid.

Table 2.3 *Todarodes filippovae.* Classification results for group membership according to year of capture from discriminant analysis on morphometric measures of mature females.

		Predicted Group Membership			
	Year of Capture	2002	2003	2004	Total
Assigned group membership	Count (%) 2002	45 (78.9)	6 (10.5)	6 (10.5)	57 (100.0)
	2003	11 (20.4)	17 (31.5)	26 (48.1)	54 (100.0)
	2004	9 (16.4)	17 (30.9)	29 (52.7)	55 (100.0)

Table 2.4: *Todarodes filippovae.* Classification results for group membership according to season of capture from discriminant analysis on morphometric measures of mature females.

			Predicted Group Membership			
		Season of Capture	Summer	Autumn	Winter	Total
Assigned group membership	Count (%)	Summer	22 (46.8)	18 (38.3)	7 (14.9)	47 (100.0)
		Autumn	35 (39.8)	46 (52.3)	7 (8.0)	88 (100.0)
		Winter	2 (6.5)	0 (0.0)	29 (93.5)	31 (100.0)

2.4.2 Relationship to SSC and SST

Todarodes filippovae specimens were obtained from waters that had marked seasonal differences in SST. In southern Tasmanian waters, average monthly summer and autumn temperatures across all years ranged from 13.1 to 15. 7 °C and 11.34 to 14.73 °C respectively (Fig. 2.4). Sea surface temperatures off eastern Tasmania were higher than in southern Tasmania by approximately 2 to 4 °C depending on the season. Consequently, winter SST off eastern Tasmania were comparable to autumn SST off southern Tasmania. During the three-year sampling period there was an observed trend of decreasing SST in southern Tasmanian waters. For example, the mid-monthly temperature for the summer season of 2002 was 15.5 °C, 14.7°C in 2003 and 13.6°C in 2004 (Fig. 2.4). Similarly, average winter sea surface temperatures in 2002 were higher (14.5°C) than for the winter of 2004 (13.0°C) off the east coast of Tasmania.

Sea surface colour for waters off eastern Tasmania consistently confirmed a seasonal cycle of chlorophyll *a* with a larger spring peak followed by a smaller autumn peak. The spring of 2001, just prior to the beginning of sampling, showed a greater amount of chlorophyll *a* than in the other years. In contrast, southern Tasmania was characterised by a more variable seasonal cycle. More specifically, the SSC profile of the study period was dominated by a sizeable peak in chlorophyll *a* in late summer/autumn of 2003 (Fig. 2.4).

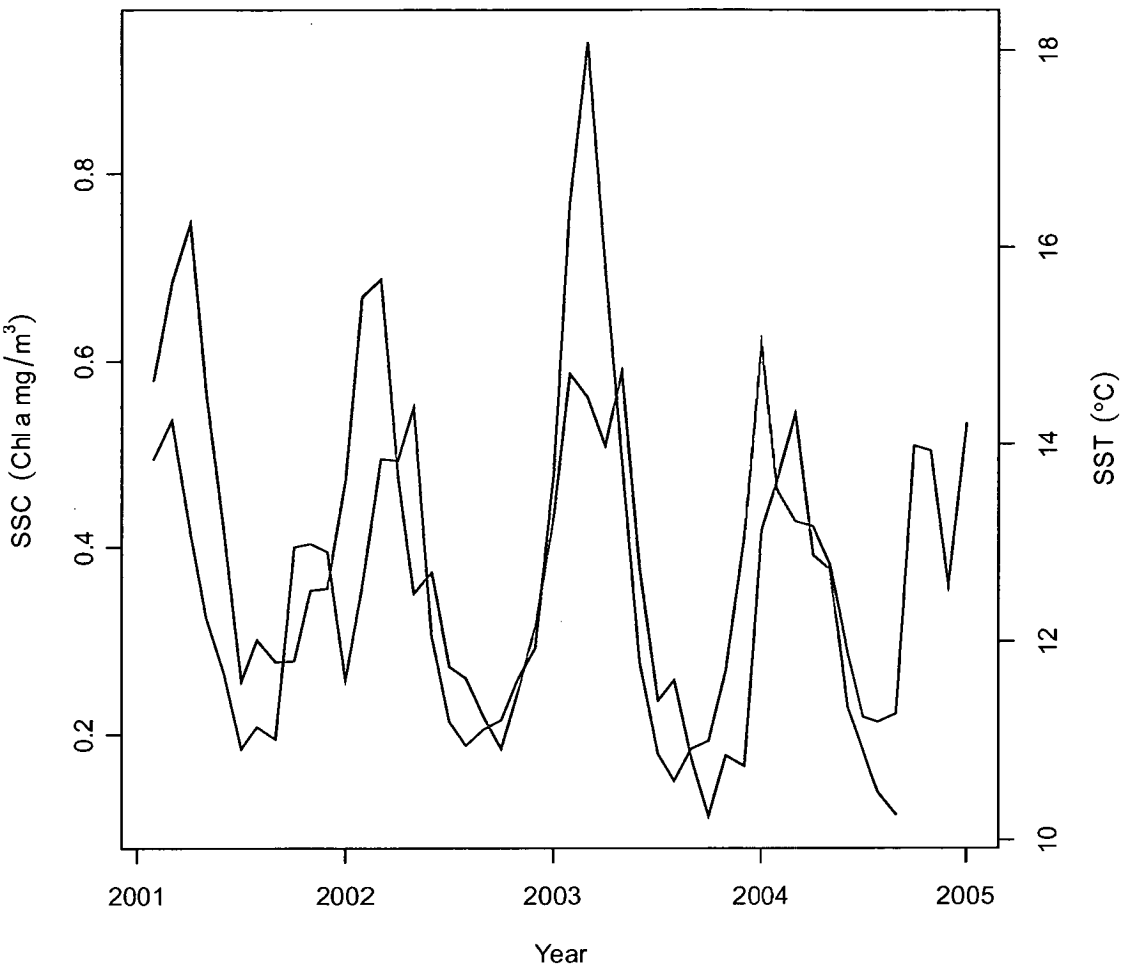

Figure 2.4: Average monthly sea surface colour (SSC, chlorophyll a concentration black line) and sea surface temperatures (SST, red line) for waters off southern Tasmania 2001-2004.

Correlation analysis was only performed on summer and autumn individuals due to the fact that only 2 winter samples were obtained off eastern Tasmania. The correlation analysis showed that gonad investment was most strongly related to SSC at time of capture, whereas condition was more strongly related to SSC two months prior. This suggests that the environmental impact on gonad investment was more immediate than the impact on condition. The relationship between the residuals and temperature was less clear. There appeared to be no positive relationship with either gonad investment or somatic condition at time of capture or in the few months prior to sampling (Fig. 2.5).

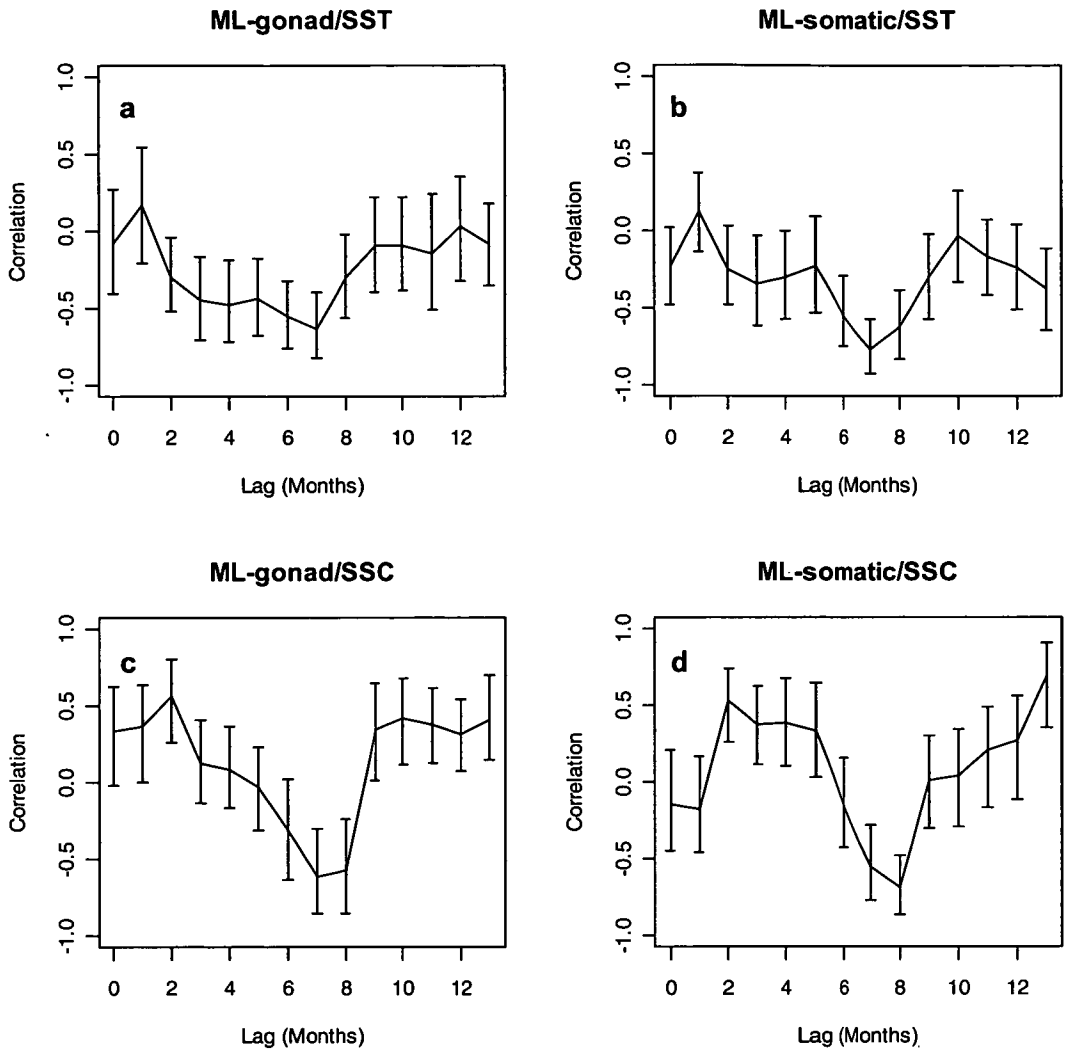

Figure 2.5: *Todarodes filippovae.* Bootstrap mean correlations and pointwise confidence intervals between lagged sea surface temperature (SST) and the residuals from the (a) ML-gonad weight regression and the (b) ML-somatic weight regression; and between lagged sea surface colour (SSC) and the residuals from the (c) ML-gonad weight regression and the (d) ML-somatic weight regression for all summer and autumn caught mature females from southern Tasmania.

2.4.3 Reproductive strategy

There were no suggestions of a trade-off for any seasonal sample in any year as indicated by the lack of any significant negative correlations between ML-somatic residuals and the ML-gonad residuals. Rather, there was a concomitant increase in both condition and gonad investment for the 2003 autumn ($r = 0.359$, $p < 0.01$, $n = 63$) and the 2004 autumn ($r = 0.354$, $p = 0.001$, $n = 89$). The 2002 autumn sample showed a significant, although weak correlation at the 0.1 level ($r = 0.247$, $p < 0.1$, $n = 48$). Similarly, the 2004 summer sample showed a concomitant increase in size-

adjusted somatic weight with size-adjusted gonad weight ($r = 0.393$, $p = 0.05$, $n = 36$).

Overall, *Todarodes fillippovae* did not have very full stomachs when captured. Across all years and seasons 76% of all mature stage 5 females had a stomach fullness of 1, indicating that a little food or only fluid was present in the stomach at time of capture. However, low amounts of food in the stomach were consistent among all maturity stages. Based on this dataset, there was no difference in stomach fullness among maturity stages, suggesting that mature stage 4 and 5 females continue to feed as per the immature and preparatory (stage 3) individuals (Table 2.5).

Table 2.5: *Todarodes filippovae*. Summary of percent stomach fullness distribution for each maturity stage of all females across all years and seasons. Stomach fullness stages range from 1 (very little) to 5 (full and distended).

Maturity Stage	Stomach Fullness Stage					Total Females
	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	
2	63.6	24.2	9.1	3.0	0	33
3	70.7	17.2	9.2	2.3	0.6	174
4	64.1	25.6	10.3	0	0	39
5	76.0	15.2	5.6	3.2	0	125

Average RSI for mature females ranged from 9.2% (2002 autumn sample) to 18.9% (2002 winter sample). The highest maximum RSI values of 29.3% and 33.1% were found in the 2002 and 2004 winter samples, along with the 2003 autumn sample that had a maximum value of 32.8%. Maximum 2002 and 2003 summer RSI values were lower than most other samples, 21% and 22.8% respectively (Table 2.6)

Both year and season were found to have no effect on the mean difference in egg batch size. Generally, there was a trend toward larger females not storing their eggs in readiness for a single spawning event as evidenced by the lack of correlation between ML and oviduct fullness for any season/year. In spite of this, females of the 2003 autumn sample did show a significant relationship between ML and oviduct fullness ($r = 0.522$, $n = 28$, $p<0.01$).

Table 2.6: *Todarodes filippovae.* Summary of reproductive parameter relationships for mature females collected from each sampling year/season, RSI=reproductive-somatic index SE=standard error.

Year	Season	Somatic and oviduct partial correlation			Ovary:oviduct ratios > 1	RSI (%)		
		r	p	n	(%)	Range	n	Mean±SE
2002	Summer	-0.496	0.258	8	0	2.83-21.03	16	11.085 ± 1.415
	Autumn	-0.866	0.026	7	0	4.11-24.5	18	9.177 ± 1.415
	Winter	-0.659	0.002	20	9.52	11.95-29.25	20	18.916 ± 1.013
2003	Summer	-0.304	0.236	18	0	5.82-22.75	21	15.178 ± 0.949
	Autumn	-0.683	<0.001	28	7.14	5.64-32.82	33	14.410 ± 1.142
	Winter	n/a						
2004	Summer	-0.680	0.093	8	0	9.46-26.7	9	14.768 ± 1.724
	Autumn	-0.495	0.009	28	0	6.81-25.94	37	13.723 ± 0.821
	Winter	-0.581	0.131	9	11.11	10.1-33.08	9	16.517 ± 2.467

On the whole, there was a tendency for increasing somatic weight to be coupled with decreasing oviduct weight for all seasons in all years. Significant negative correlations $p<0.05$ (when controlling for BW) were found for all autumn samples and the 2002 winter sample, while the summer sample of 2004 was significant at the 0.01 level (Table 2.6). There was also very little evidence that females were depleting their ovaries of oocytes as they were transferred to the oviducts in preparation for spawning. All summer samples over the three years and two autumn samples recorded no ovary:oviduct ratios >1. Both winter samples and the 2003 autumn sample showed some evidence of the ovary being depleted. However, no more than 12% of the samples had ovary:oviduct ratios >1 (Table 2.6).

Only when female individuals of *T. fillippovae* reached stage 3 maturity was there any indication of prior mating, as evidenced by spermatophores in the buccal area or stored sperm in the seminal receptacles. However, among all years and seasons there was wide variability in the percentage mated in the preparatory stage (stage 3). In the summer of 2004 only 16% had mated while in the summer of 2003, 77.7% had mated. Interestingly, it was only in the autumn samples that not all of the maturing females (stage 4) had mated. Similarly, it was only in the autumn 2003 and 2004 samples that not all of the mature (stage 5) females had mated (Fig. 2.6).

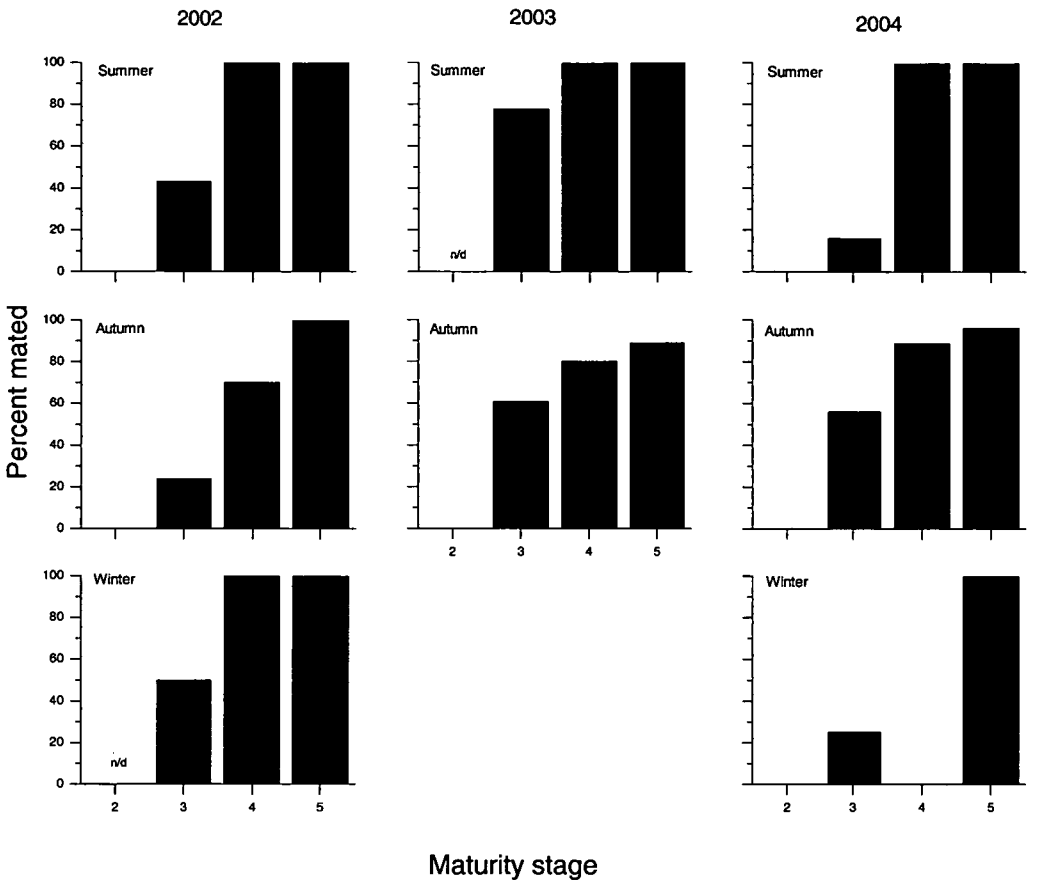

Figure 2.6: *Todarodes filippovae.* Summary of percent mated females for each maturity stage for all year/season combinations.

2.5 Discussion

This three-year study revealed that female individuals of *T. filippove* from Tasmanian waters express extreme plasticity in their reproductive tactics resulting in flexible life history patterns. Female individuals of *T. filippovae* exhibited both annual and seasonal variation in somatic condition coupled with variation in gonad investment that appeared to be principally driven by a changeable environment. Furthermore, the reproductive strategy of this species highlighted an opportunistic lifestyle, whereby multiple spawning was employed to exploit opportunities that may ensure hatchling survival in an unpredictable environment. This marked plasticity in life history patterns characterizes the responses already reported for some other squid species (e.g. Villanueva, 1992; Boyle et al., 1995; Jackson et al., 1997; Hatfield,

2000; Jackson et al., 2003; Pecl et al., 2004). These differing patterns in cephalopod populations may be predominantly influenced by phenotypic plasticity (Boyle & Boletsky, 1996) in response to oceanographic conditions peculiar to the region, the inherent changeability in oceanographic parameters and the overall biological response to the changeability (Anderson & Rodhouse, 2001).

2.5.1 Oceanography of study region

Todarodes filippovae in this study were caught in a region where there is both seasonal and annual variability in oceanographic conditions. In any ecosystem, nutrient fluctuations are the driving factors influencing primary productivity (Prince, 2001). The most influential source of nutrients in this region is the deep oceanic sub-Antarctic waters (SAW). The seasonally mixed layers of SAW off southern Tasmania, typical of temperate regions throughout the world, are characterised by a spring and autumn bloom. Although the Zeehan Current (ZC), an extension of the surface flowing Leuwin Current, sometimes influences the south west of Tasmania with intrusions of oligotrophic sub-tropical waters (Harris et al., 1991; Prince, 2001). The waters off eastern Tasmania are dominated by a complex hydrography (Young et al., 1996a). The warm stratified nutrient depleted East Australian Current (EAC) that flows southward along the east coast of Tasmania meets the cooler, often deeply mixed, nutrient rich sub-Antarctic waters (SAW) that forms the sub-tropical convergence (STC) (Harris et al., 1991, Young et al., 1996b, Prince, 2001). The STC, an extensive region of enhanced oceanic productivity (Marshall, 1979 cited in Prince, 2001) shifts seasonally and annually around southeastern Tasmania (Harris et al., 1987) due to the interaction between the EAC and SAW; this shifting subsequently determines both seasonal and interannual variation in productivity levels (Young et al., 1993). Furthermore, individuals of *T. filippovae* in this study were caught in what the fishermen call fishing 'hotspots'. In waters off southern Tasmania these hotspots include the Maatsuyker seamounts, and the 'St Helens Hill' seamount off eastern Tasmania. These waters are characterised by a combination of oceanographic and topographic features that drive the seasonal variability of these ecosystems (Prince, 2001).

2.5.2 Temporal variability in condition and gonad investment

Squid are typically opportunistic therefore enabling them to respond rapidly to changes in the environment (Rodhouse, 2001). It is this rapid response in growth and reproductive patterns to changing environmental conditions, whether they be deleterious or favourable, that has led to squid being considered 'environmental indicators' (Jackson & Domeier, 2003). Although the consistent results found between the residuals analysis and the discriminant analysis highlighted the temporal variability in condition and gonad investment in this species, it is often difficult to determine any underlying cause due to the complexity of the influence of biotic and abiotic factors. Nevertheless, positive correlations between both gonad investment and somatic condition with SSC suggested that differences in these parameters may be causally related and therefore account for some of the temporal variability observed in this study. It cannot be discounted that the correlations were simply reflecting seasonality in the data and that if other factors were being controlled for, this trend may not be evident. A lag of approximately 6 months resulted in evidence (dip in negative correlations) of seasonality in all correlation analyses. However, given that SST and SSC displayed similar trends over the sampling period, a similar result for both SST and SSC when correlated with condition and gonad investment would be expected. On the contrary, no positive relationships were observed with SST.

Much of the variation attributed to the positive relationship between lagged SSC and condition may be due to the peak in SSC in late summer/autumn of 2003. The two - month lag observed in the correlation analysis coincided with the highest level of condition during the study period for squid caught off southern Tasmania. The poorer condition of animals found in the first year of the study may be due to comparatively lower SSC and higher temperatures in that year than in 2003. Warmer nutrient poor waters resulting from the EAC or ZC on the west coast may have been more influential in southern Tasmania in 2002 and the latter part of 2001. However, the relationship between condition and SSC in 2004 is less clear. Although SSC was marginally higher in 2004, than in 2002 when condition was poor, it was much lower than 2003. This suggests that the higher condition also found in 2004 relative to 2002 may be due to other factors, or perhaps food was not limited as it may have been in the first year. It is noteworthy that even though no relationship between SST and

condition was found, 2004 was the coolest year (by up to 2°C). The role of temperature in this study remains unexplained. It may be that a signal for temperature was not detected in this study due to the limited seasonal sampling (i.e., only summer and autumn from southern Tasmania). Moreover, under certain conditions the importance of one variable may override another. It has been suggested that juvenile squid growth is only constrained by temperature prior to primary production blooms but is likely to be limited by food resources after the bloom peaks (O'Dor, 1998).

Results of this study are consistent with that documented for *Sepioteuthis australis.* This species also exhibited temporal variation in condition, with summer and spring-hatched individuals in better condition than winter or autumn hatched animals. Differences in condition and growth were largely attributed to variation in regional oceanographic variables (Pecl 2004). In the present study the two-month lag observed between SSC and condition suggests that mature females may be benefiting from a downstream effect in secondary production and from the associated concentration of prey species. It is expected that the fluctuating nature of targeted prey species may be partially deterministic in defining life history strategies of marine organisms (O'Dor, 1998). For the Northeast Arctic cod, temporal and annual variation in condition was linked to the abundance and availability of capelin and herring in the Barents Sea (Yaragina & Marshall, 2000). Furthermore, the collapse of the jack mackerel fishery off eastern Tasmania in 1989 has been attributed to the absence of normally abundant krill stocks in that year. The absence of krill stocks coincided with an intrusion of EAC nutrient-poor sub-tropical waters in the austral summer-autumn when krill stocks are usually abundant (Young et al., 1993). Myctophids, an important component of the diet of *T. filippovae* (Pethybridge, 2004) also exhibits seasonal fluctuations in abundance off eastern Australia (Prince, 2001). Although temporal changes in diet were not found in *T. filippovae* caught between March and July 2004 off Tasmania (Pethybridge, 2004), a longer-term study is needed to reflect availability and abundance of prey.

The positive correlation between SSC and gonad investment readily explains annual differences between the poorer year of 2002 compared to 2003. However, gonad investment was even higher in 2004 than in 2003 that exhibited the marked peak in

SSC. As suggested for variation in condition, reproductive investment is most likely not due to any one factor but to the complex interaction between biotic and abiotic factors. A two-year study investigating reproductive plasticity in *Nototodarus gouldi* found that gonad investment varied according to location, with higher latitudes tending towards greater gonad investment (McGrath-Steer, 2004). However, in a separate study (Portland, Victoria), gonad investment by female individuals of *N. gouldi* was found to vary temporally, with lowest investment in the cooler months of May to July (McGrath-Steer & Jackson, 2004). In contrast, in this study female individuals of *T. filippovae* caught in the winter months showed significantly higher gonad investment than specimens caught in summer and autumn. However this is not inconsistent, as the mechanism driving the differing seasonal responses between these two ommastrephid species may be similar. McGrath-Steer and Jackson (2004) suggest that the months of greatest gonad investment coincided with periods of higher productivity due to the Bonney Upwelling off the coast of Victoria, Australia. Similarly, higher reproductive investment in *S. australis* in some years was attributed to corresponding cooler more productive years (Pecl et al., 2004). Since the temperatures off eastern Tasmania in winter were similar to those in autumn off southern Tasmania, it is unlikely that temperature was a principal factor in the seasonal variation in gonad investment in this study. Moreover, both samples of winter-caught squid (no sample collected in 2003) were predominantly all mature mated individuals, indicating that during winter the waters off eastern Tasmania were a spawning ground for *T. filippovae*. This is in contrast to specimens caught off southern Tasmania in the winter of 2004 where 80% were immature (Jackson et al., in prep). The winter caught individuals off eastern Tasmania, were investing more in reproduction and spawning appears timed to coincide with periods of increased productivity. The retreat of the nutrient poor EAC in winter (Young et al., 1996b) leaves behind richer SAW characterised by a relatively larger spring bloom than autumn bloom (National Oceans Office, 2002), thus providing enriched conditions for enhancing hatchling survival. Similarly, it has been suggested that in gemfish, egg and larval production coincides spatially and temporally with phytoplankton blooms off Eastern Australia (Prince, 2001).

Long-range migrations of many commercially important ommastrephids are associated with large oceanographic current systems (O'Dor, 1992; Boyle et al.,

1995). Although there has been no evidence to suggest mass migration in *T. filippovae*, it is possible that small-scale migrations may occur in relation to spawning events. It has been suggested that *T. filippovae* may move to continental slope waters to spawn and that this may occur towards the northern boundary of its distribution in the Tasman Sea region (Dunning, 1993). Mature mated individuals were found in all samples in southern and eastern Tasmania and therefore it seems possible that in addition to the spawning region of 'St Helens Hill', off Eastern Tasmania, spawning may occur in a number of slope and seamount regions across its distributional range. The relatively smaller size of the winter caught squid (Jackson et al., in prep) may suggest, as observed for *N. gouldi* (Jackson et al., 2003) that these individuals may be a different morph that have moved into or are resident populations of the seasonally richer SAW. However, it must be noted that two mature specimens caught in southern Tasmanian waters were used in the 2004 winter sample and no major size differences were found. It is possible that these two specimens may not be representative of the southern population. Alternatively, the size differences may be purely due to temporal differences in growth as reported for other winter-caught squid species (Jackson & Moltschaniwskyj, 2002; Ichii et al., 2004). Further research involving seasonal samples from both southern and eastern Tasmania is needed to determine the life history characteristics of the populations from these two regions.

2.5.3 Maternal effects

In this study, and a five-year study conducted on *S. australis* off eastern Tasmania (Pecl et al., 2004), better condition was coupled with increased gonad investment. Variation in the amount of energy available for competing physiological processes is dependent on the acquisition of resources (Van Noordwijk & De Jong, 1986). Any trade-off between available food resources or energy reserves and gonad investment may involve various tactics. Reproductive investment may be maintained at the expense of somatic condition, which would likely increase maternal mortality but increase hatchling survivor rate. Alternatively, lower gonad investment (resulting in lower fecundity) may limit the loss of somatic condition, or maturation may be delayed in extreme circumstances (Lambert & Dutil, 2000). A study on female cod showed that those females in poor condition had a greater relative loss in somatic

condition post-spawning than those in good condition. Furthermore, fecundity and total egg dry weight was significantly less in females of poorer condition (Lambert & Dutil, 2000). Unlike some fish that store energy for maturation and spawning (Hernandez et al., 2003, Craig et al., 2000, Lambert & Dutil, 2000), *T. filippovae* appears to fuel the maturation process by direct food acquisition. Therefore, resource-poor environments are likely to be highly influential on maternal condition, and subsequently maternal effects. Steer et al. (2004) in an experiment using the sepiolid *Euprymna tasmanica* found that low maternal food ration resulted in smaller clutches and smaller egg size coupled with increased mortality rates. Under food stress, the sepioid *Idiosepius pygmaeus*, maintained frequency of egg laying and egg size but laid fewer eggs compared to females with unlimited food supply (Lewis & Choat, 1993). Feeding regime in *Loligo pealeii* failed to show any significant influence on reproductive output or reproductive index (Maxwell & Hanlon, 2000). Although maternal condition was not measured in these experiments it is evident that cephalopods will apply various reproductive strategies when under food stress. *Todarodes filippovae* was able to maintain egg batch weight inter-annually and seasonally regardless of condition or reproductive investment. Theoretically it was expected that squid investing more in gonads would also produce larger batches of eggs. However, it is not known whether a trade-off between egg size and fecundity was occurring. The 'bigger is better' hypothesis suggests that larger eggs would produce larger hatchlings which infers a survival advantage due not only to a greater ability to catch prey and avoid predators, but also to the ability to sustain periods of low productivity (Rideout et al., 2005). In the present study, a cost associated with egg batch size and female condition was detected, as females with larger egg batches tended to be in poorer condition. This was particularly evident in all autumn samples and the 2002 winter sample. This evidence suggests that for these populations a trade-off was occurring between somatic condition and egg batch weight.

Extreme variation in annual recruitment has been noted for many marine species (Boyle & Boletsky, 1996). In short-lived squid, both the physical and biological environment of the spawning grounds is probably highly influential in determining recruitment success (Sakarai et al., 2000; Waluda et al., 2001). Since there is an intimate relationship between condition and gonad investment for *T. filippovae* it is expected that the interplay between these biological parameters and environmental

variation may be highly influential in determining maternal effects (e.g. egg size and fecundity), which in turn affects survival and perhaps ultimately abundance and recruitment (see Heyer et al., 2001, Oullet et al., 2001). In fish, condition has contributed to population success (Lloret et al., 2002), as indicated by the positive association with hatching success and survival of herring in the Baltic Sea (Laine & Rajasilta, 1999). Furthermore, in haddock, condition has been used as a proxy for total reproductive potential and positively correlated with recruitment success over three decades (Marshall & Frank, 1999). Moreover, under experimental conditions, a resource rich environment enabled both small and large larval fish to feed successfully and achieve similar growth and survival, but in a resource poor environment smaller larvae were disadvantaged (Rideout et al., 2005). Fish larvae in poor condition were also found to have a lower response rate to a model predator due to developmental delay and reduced energy reserves to allocate to avoidance of predators (Skajaa et al., 2004; see also Elliott & Leggett, 1998). Similarly, in *Illex* schools deprived of food, smaller weaker and less aggressive squid were canabalized in three days in captivity (O'Dor, 1998). The impact of small changes in the mortality rate of juveniles consequently has immense effects on adult recruitment (Pecl, 2004). An emerging pattern in cephalopods is that there is protracted spawning year-round with peaks associated with high productivity (O'Dor, 1998). It is highly likely that the relationship found between environmental predictors and recruitment and abundance of squid (see Robin & Denis, 1999; Yatsu et al., 2000; Agnew et al., 2002; Waluda et al., 2004) may in part be related to maternal condition and maternal effects in response to environmental conditions.

2.5.4 Spawning strategy

Research is increasingly indicating that multiple spawning is becoming a frequent life history pattern displayed by squid (eg; *Loligo forbesi*, Boyle et al., 1995, Collins et al., 1995; *Photololigo* sp, Moltschaniwskyj, 1995; *L. vulgaris reynaudii*, Melo & Sauer, 1999; *L. pealeii*, Maxwell & Hanlon, 2000; *S. australis* off Tasmania, Pecl 2001) including ommastrephid species (*Stenoteuthis oualaniensis*, Harman et al., 1989; *Nototodarus gouldi*, McGrath & Jackson, 2002). A lack of a strong correlation between oviduct fullness and body size suggested a multiple spawning strategy for *T. filippovae*. This implies that females were not storing their eggs over long time

periods in preparation for a single egg batch as would be expected for a terminal spawning squid (Harman et al., 1989; Moltschaniwskyj, 1995; Pecl, 2001). Further confirmation for this strategy was due to a lack of strong evidence supporting ovary depletion following transfer of eggs into the oviducts, since it would be reasonable to assume that oviduct weight would surpass ovary weight if eggs were accumulating for a terminal event (Moltschaniwskyj, 1995). An exception is *Moroteuthis ingens* that retains all mature eggs in the ovary, using the oviduct as a passage when spawning without any accumulation time for their massive spawning event (Jackson et al, 2004).

It has been suggested that the type of reproductive strategy adopted for a given species may fall somewhere along a continuum (Jackson & Mladenov, 1994), whereby within the strategy adopted there is inherent flexibility due to environmental constraints. The correlation between ML and oviduct fullness coupled with some evidence of ovary depletion for autumn 2003 suggests that the females caught at this time may be shifting towards the terminal end of the continuum. In addition maximum RSI values for this sample were relatively high (32.82%), as they were for both 2002 and 2004 winter samples (29.25 and 33.08% respectively). In contrast, maximum values for summer samples ranged from 21 – 26% over the three years. Although the average RSI of *T. filippovae* females were only slightly higher than that found for other ommastrephids (e.g. 8% in *S. oualaniensis*, Harman et al., 1989; 5 – 10 % in *Dosidigas gigas*, Markaida & Sosa-Nishizaki, 2001; 8 – 15% in *N. gouldi*, McGrath-Steer, 2004) they were considerably lower than that recorded for an ommastrephid (e.g., up to 50% for individual *Todarodes pacificus*, Ikeda et al., 1993), loliginid (e.g., 25-50% for *Loligo opalescens*, Fields, 1965), or onychoteuthid (eg. 26% for *M. ingens*, Jackson et al., 2004) species more towards the terminal end of the continuum.

Females tend to invest relatively greater amounts of energy than males in the reproductive process due to greater growth of the organs involved. Reproductive investment in females of this species appears to be highly plastic. McGrath-Steer (2004) also found extreme plasticity in the ommastrephid *N. gouldi* and suggested that environmental conditions may be important in determining the amount of energy available for maturation. It is likely that the multiple spawning strategy of these *T.*

filippovae females is inherently plastic resulting in a population shift along the multiple versus terminal continuum depending on the conditions experienced at the time of maturation.

Theoretically, reproduction involves a cost in terms of growth and survival, and potential fecundity (Forsman, 2001). Many marine species, such as fish (e.g., Poizat et al., 1999; Abitia-Cardenas et al., 2002), krill (Nicol et al., 2004), seastars (Raymond et al., 2004) and some squid (e.g. Jackson & Mladenov, 1994, Arkhipkin & Bjorke, 1999) store energy that is later sequestered for reproduction resulting in a reproductive-somatic trade-off. At the whole animal level there was no evidence in this study to suggest that energy from the soma was being mobilised to fuel the reproductive process in females of this species. A lack of a reproductive-somatic trade-off (or very limited cost) has also been found in other multiple spawning squid species (eg. *S. oualaniensis*, Harman et al., 1989; *L. gahi*, Guerra & Castro, 1994; *Photololigo* sp, Moltschaniwskyj & Semmens, 2000). In studies on *N. gouldi* from Tasmanian waters (McGrath & Jackson, 2002) and over broad geographical and seasonal temporal scales in southeastern Australian waters (McGrath-Steer 2004) no reproductive-somatic trade-off was detected. However, in a subsequent study by McGrath-Steer and Jackson (2004) investigating temporal shifts in the allocation of energy of *N. gouldi* from southern Australian waters, a reproductive-somatic trade-off in females was attributed to specific environmental conditions that may force plasticity as seen in this species when energy is partitioned between competing physiological processes. They suggest that a trade-off may be unlikely under resource rich conditions but more likely under resource poor conditions. It is most likely that female individuals of *T. filippovae* derive their energy requirements for maturation through the direct acquisition of food as suggested for *Sepioteuthis australis* (Ho et al., 2004), *Photololigo* (Moltschaniwskyj & Semmens, 2000), *N. gouldi* (McGrath & Jackson, 2002), *Dosidigas gigas* (Markaida & Sosa-Nishizaki, 2001) and *Illex argentinus* (Hatfield & Rodhouse, 1992).

The concomitant increase in both somatic and reproductive processes in all autumn samples of *T. filippovae* may be due to seasonal variation in productivity in southern Tasmanian waters. The higher primary productivity in autumn may facilitate a strategy whereby females do not have to preferentially allocate resources to

reproduction over somatic growth. Reproduction in squid appears to be timed so as to allow hatchlings to take advantage of production events such as blooms (O'Dor, 1992). Ommastrephid rhynchoteuthian hatchlings seem to initially feed on particulate matter and microorganisms trapped or growing on the mucus covering of the hatchlings. This enriched mucus is probably used as a food source for early rhychoteuthians (Vidal & Haimovici, 1998). However, once juveniles are able to capture prey, the voracious feeding and rapid growth of a squid cohort enables them to track a primary production peak through time, as energy moves up the trophic levels (Dawe & O'Dor, 1998; O'Dor, 1998).

The stronger correlations between weight of the reproductive structures and size as opposed to age suggested that the maturation process was more closely governed by size regardless of age. Poorer environmental conditions may delay maturation. Extended periods of slow growth before the minimum body size for maturation is reached (Jackson, 2004) may partially be a direct result of feeding conditions.

2.6 Conclusions

The plasticity in condition and reproductive parameters exhibited by female individuals of *T. filippovae* highlights that this species is capable of exploiting variable environmental conditions. Dunning (1988b) suggests that small-scale, local events and topographic features such as seamounts are more likely to influence the distribution and lifecyle of ommastrephid species in Australian waters. These oceanographic conditions are likely to result in geographical and short-term areas of productivity. This is in comparison to the large-scale permanent oceanographic features and subsequent areas of increased biological productivity experienced by some other ommastrephid species in other parts of the world. Therefore, the life history of ommastrephids in this oceanographic region may in fact be intrinsically more complex and variable (Dunning, 1988b). However, it is possible that highly variable environments may offer unseen advantages that outweigh or counterbalance any associated costs. Fish populations experiencing greater environmental fluctuations actually were found to have similar or higher condition factors, and more robust individuals than conspecifics inhabiting more stable environments (Spranza & Stanley, 2000). The opportunistic lifestyle of squid enables them to adopt strategies to exploit opportunities when and wherever they occur, which in turn results in

variable life history patterns. Multiple spawning is likely to be one such tactic, which enables females to produce hatchlings at varying intervals thus ensuring the success of some batches (Sauer et al., 2002). In extremely variable environments this risk spreading strategy ensures that at least some hatchlings will likely encounter optimum conditions for growth and survival.

CHAPTER THREE

UNDERSTANDING REPRODUCTIVE PARTITIONING IN *TODARODES FILIPPOVAE* – A LIPID AND FATTY ACID APPROACH

3.1 Abstract

The role of lipid in relation to maturation was examined in female individuals of the ommastrephid *Todarodes filippovae*. Squid were obtained from the commercial deepwater fishery during autumn and winter in waters off southern and eastern Tasmania during 2004. At the whole animal level there was no evidence of energy being diverted from the soma from either the digestive gland or mantle for maturation. However, principal component analysis revealed that as females matured, there was a concurrent increase in mantle and ovary lipid but a corresponding decrease in digestive gland lipid. The lack of variation between digestive gland lipid content and mantle lipid content between immature and mature individuals suggests that the tradeoff was limited. Lipid content increased significantly in the ovary with maturation ($p<0.001$). There were marked differences in the lipid classes and fatty acids with maturation that was suggestive of their importance in embryonic development, and by inference to the rhynchoteuthion. The gonads were predominantly phospholipid (up to 90%), followed by sterol (7-12%) with minor amounts of reserve lipid. The fatty acid profile was dominated by saturated fatty acids (40%). Polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids accounted for 35% and 25 % of total fatty acids respectively. PUFA was dominated by the essential fatty acids DHA and EPA. Differences in lipid class and fatty acids between the ovary and oviduct were comparatively minimal and not consistent between seasons. The ovary of mature autumn females had significantly greater lipid content than mature winter females ($p<0.05$). There was an overall greater provisioning of polar lipid and less neutral lipid in the oviduct of autumn females compared to winter females. However, the quality of the oocytes in the ovary and the ovulated egg in the oviduct in terms of the fatty acid profile was relatively conserved between seasons. The role of lipid in maturation of *T. filippovae* is not as a major energy reserve but one of maternal provisioning of essential nutrition to the developing embryo. Any observed temporal differences in the ovary and oviduct may simply reflect reproductive plasticity in response to the environment.

3.2 Introduction

Pelagic squids are successful competitors and ecological equivalents to teleost fish in many marine environments. However, relative to their fish counterparts, squid have a markedly dissimilar pace of life in terms of physiology and metabolism (O'Dor & Webber, 1986). Furthermore, these poikilothermic animals have exceptionally elevated growth rates that are more synonymous to homeothermic, terrestrial animals (Lee, 1994). This extraordinary growth is in the most part due to their voracious carnivory, efficient and rapid digestion in association with a predominantly protein/amino acid metabolism (O'Dor & Webber, 1986; O'Dor & Wells, 1987). Given that growth and maturation are coupled (Mangold et al., 1993), the processes that are used to fuel maturation are of considerable interest. In marine organisms reproduction is often intrinsically tied to the mode of energy storage used to fuel oogenesis. Such storage is often essential, but constrained by the finite ability of an organism to partition available resources between competing physiological processes (Jackson et al., 2004; McGrath-Steer, 2004). As a consequence, many fish and invertebrate species have adopted various reproductive strategies involving energy partitioning for maturation, overwintering and spawning (e.g. Falk-Petersen et al., 2000; Lambert & Dutil, 2000; Hernandez et al., 2003). These strategies help reduce the associated reproductive costs in terms of future fecundity for the female; as well as both maternal and hatchling costs in relation to growth and survival (Forsman, 2001). Carbon-rich lipids are high in energy value and as such are important metabolic fuels for many marine organisms (Parrish et al., 2005). Falk-Peterson et al. (2000) suggest that the fundamental use of accumulated neutral lipid in marine organisms is to serve as an energy reserve. They also suggest that in many marine animals neutral lipid may serve as a buoyancy mechanism. Despite the fact that squid have a predominantly protein based metabolism, the large neutral lipid-rich digestive gland has been cited as a key storage substrate for maturation and spawning in some species of squid (Nesis, 1987; Arkhipkin & Bjorke, 1999; Seibel et al., 2000).

The digestive gland is associated with a number of digestive processes, including enzyme synthesis and secretion, nutrient absorption, nutrient storage and excretion of waste (Boucaud-Camou et al., 1985; Nesis, 1987; Mangold et al., 1998; Semmens et al., 1995; Semmens, 1998; Swift et al., 2005). Recorded levels of lipid content in the digestive gland may range from 8% wet mass in the cuttlefish *Sepia officinalis*

(Blanchier & Boucaud-Camou 1984) to 41.7± 8.5% wet mass in the digestive gland of the terminal spawning squid *Moroteuthis ingens* caught around Heard Island (Phillips et al., 2001). This is in marked contrast to lipid content in the mantle that is generally less than 1-2% wet weight (Lee, 1994; Phillips et al., 2001). These large deposits of lipid in the digestive gland have led to much conjecture regarding its role as a storage organ for reproduction. Evidence supporting some mobilisation of lipid as an energy source comes from experimental trials where lipid content in the digestive gland has decreased following starvation (O'Dor et al., 1984; Castro et al., 1992). However, as has been suggested by Semmens (1998), without further replenishment of food, much of the lipid in the digestive gland may have simply been excreted over a number of days. This was based on later research that showed excess non-structural lipid (at least in loliginid squid) was excreted not stored in the digestive gland (Semmens, 1998). Furthermore, O'Dor et al. (1984) suggested that in comparison to protein, lipids were limiting to growth. This was due to slow absorption and the restricted metabolism of this energy rich nutrient, as evidenced by the oily faeces of cephalopods fed high lipid diets. There is support for the catabolism of protein in the mantle as a source of energy in reproduction as evidenced by the increase in acid protease activity and thinning of the mantle in conjunction with maturation in the ommastrephid *Todarodes pacificus* (Shikata & Shirata, 1999). Similarly, in the terminal spawning squid *Moroteuthis ingens*, thinning of the mantle coupled with the complete loss of mantle muscle, was associated with the mobilisation of protein as energy fuel for oogenesis (Jackson & Mladenov, 1994; Jackson et al., 2004).

Despite the apparent limited lipid requirements by squid, a pivotal paper by Navarro and Villanueva (2000) underscored the importance of lipid in the diet of hatchling cephalopods for optimum growth and survival. Lipids are critical for the functioning of a number of biological processes in organisms including, as structural components (predominantly phospholipids) of cell membranes, as precursors for chemical messengers and as a catabolic reserve (Tveiten et al., 2004). Substantial work on the manipulation of broodstock diets in teleost fish have highlighted that the provisioning of lipid into the developing egg is a consequence of maternally derived nutrition, the mobilisation of body lipid as well as *de novo* synthesis (Mourente et al., 2002). Certain fatty acids such as polyunsaturated fatty acids (PUFA) are essential

nutrients for marine animals including cephalopods (Navarro & Villanueva, 2000; Koueta et al., 2002; Parrish et al., 2005). The fatty acids belonging to the n-3 and n-6 series are particularly imperative (Wiegand et al., 2004) due to the restricted ability of marine animals to synthesize long chain ($\geq C_{20}$) PUFA from short chain PUFA (Bell & Sargent, 2003; Bell et al., 2003; Troedsson et al., 2005). Therefore, these long chain PUFA are primarily obtained from the diet (Troedsson et al., 2005). The importance of the essential fatty acids (EFA) from the n-3 series (Docosahexaenoic acid (DHA) 22:6(n-3); Eicosapentaenoic acid (EPA) 20:5(n-3)) was highlighted in a study of juvenile cuttlefish reared for 30 days under differing feeding regimes that resulted in faster early juvenile growth. This growth benefit was conserved over the study period (Koueta et al., 2002). Furthermore, in fish the requirements of these EFA have been shown to be species specific and intrinsically tied to life history strategies (Sargent et al., 1999).

The lipid requirements of squid during embryonic development are essentially unknown. However, recent research by Rosa et al (2005) highlighted the dominance of PUFA, in particular DHA and EPA, in the gonads of the ommastrephid squids *Illex coindetii* and *Todaropsis eblanae*, thus implicating their significance in the growth of the embryo. Although some squid species have well developed hatchlings that are miniatures of the adults, this is not true for the family ommastrephidae. On the contrary, ommastrephid hatchlings have a distinct and quite small rhynchoteuthion 'larval' form that is defined by fusion of the tentacles into a proboscis. The splitting of the proboscis takes place at between 6-10 mm ML (Wormuth et al., 1992) while hatching size is from <1mm to 2mm (Watanabe et al., 1996). Furthermore, while the hatchlings of many squid species possess sizeable external yolk reserves, such reserves in rhynchoteuthions are rudimentary (Boletzky, 2003). The transition from hatching to exogenous feeding is likely to be a critical period, particularly in rhynchoteuthions due to their unusual and unique mode of feeding. Rhynchoteuthions appear to feed passively, predominantly on particles and microorganisms that are collected on their mantle mucous and transported to their mouth possibly by the action of cilia or by cleaning behaviour. The mantle mucous may also act as a substrate for microbial growth (O'Dor et al., 1985; Vidal & Haimovici, 1998).

The overall aim of this component of the research was to explore the lipid dynamics as it relates to maturation in an oceanic ommastrephid squid species. While many species to date have been shown to directly acquire energy from food for oogenesis (e.g., *Nototodarus gouldi*, McGrath & Jackson, 2002; *Dosidigas gigas*, Markaida & Sosa-Nishizaki, 2001) and still other species have exhibited a breakdown in mantle protein tissue to fuel reproduction (e.g. *Moroteuthis ingens*, Jackson & Mladenov, 1994), there has been no direct evidence for the use of lipid in the digestive gland as a reproductive reserve. Therefore, it was of interest to assess the role of lipid in *Todarodes filippovae* as a storage substrate to fuel maternal reproduction, particularly in relation to the digestive gland. Secondly, in this study, the lipid profile of both the mature ovary and ovulated eggs in the oviduct was used to infer the importance of particular lipids and fatty acids (i.e. essential fatty acids) as they relate to the embryo and potentially the rhynchoteuthion. Furthermore, the lipid class profile was used to determine the presence or absence of any energetic reserves in the form of neutral lipid in the egg that may assist the rhynchoteuthion through a critical transition to exogenous feeding. Finally, in view of the fact that female squid caught in winter had higher gonad investment than their autumn counterparts (see chapter 2), it was of interest to examine seasonal lipid profiles with respect to egg quality.

3.3 Methods

3.3.1 Collection details

Squid samples were collected during the austral autumn (March to May) and winter (June to July) in 2004 from the commercial deepwater trawl fishing industry in waters off southern and eastern Tasmania. Specimens were frozen at -20°C on board ship soon after capture. Subsequent to defrosting, dorsal mantle length (ML, mm) was recorded for each individual, along with other morphometric data including mantle wt (MW, g), digestive gland weight (DGW, g), ovary weight (OW, g) and oviduct weight (OVW, g). Additionally, each specimen was assigned a maturity stage relative to the colour and size of the reproductive organs (after Lipinski, 1979). Based on maturity stage each specimen was then assigned as either immature (up to and including stage 3) or mature (stage 4 & 5).

3.3.2 Lipid extraction and fatty acid analyses

A small amount of ovary and oviduct tissue (~ 0.5 g) was dissected from the mid-region of each organ. Similarly, approximately ~1cm of mantle tissue was collected from the mid-dorsal region of the squid mantle. Digestive gland (~ 1g in most cases) was sampled from the mid-region of the gland, and in all but 4 cases mixed with a hand held blender or spoon. All tissue samples were subsequently frozen in liquid nitrogen before being stored in a –80°C freezer prior to lipid extraction.

All tissue samples were quantitatively extracted overnight by way of a modified Bligh and Dyer (1959) one-phase methanol-chloroform-water extraction (2:1:0.8 v/v/v). The following day the phases were separated by the addition of chloroform and water (final solvent ratio, 1:1:0.9 v/v/v methanol:chloroform:water). The separation of an upper acqueous layer and lower chloroform phase was formed after ~ 4 to 5 hours. Solvents were then removed from the lower chloroform phase via roto-evaporation (~ 40°C) to acquire the total solvent extract (TSE). The TSE was weighed to provide total lipid content (% and mg/g wet mass) and determined gravimetrically. TSE for all samples were then prepared to a known volume with chloroform and subsequently stored under refrigeration prior to lipid class and fatty acid analyses.

An aliquot (~1 µl) of TSE was analysed in duplicate by way of an Iatroscan MK V TH10 thin layer chromatography-flame ionization detector (TLC-FID) analyzer (Tokyo, Japan) to determine individual classes for each sample. A polar system (60:17:0.1 v/v/v hexane:diethyl ether:acetic acid) was used to resolve the lipid classes (Volkman & Nichols, 1991) in the mantle, ovary, oviduct and digestive gland samples. For the digestive gland samples, a non-polar system (96:4 v/v hexane:ether) was additionally used to separate hydrocarbon from wax esters and diacylglyceryl ethers from triacylglycerols. Quantification of lipid class peaks was performed using DAPA Scientific Software (Kalamunda, Western Australia). A proportion of the lipid content and lipid class analyses on digestive gland and mantle were conducted during a companion study examining the diet of *T. filipovae* (Pethybridge, 2004).

An aliquot (50µl) of the TSE was transmethylated with a mixture of methanol:hydrochloric acid:chloroform (10:1:1 v/v/v) at 100 °C for 2 hours to

produce fatty acid methyl esters (FAME). FAME were separated by the addition of 1ml of water and then extracted with hexane and chloroform (4:1 v/v, 3x1.5 ml). After concentrating FAME using nitrogen gas, FAME were silyated by the addition of 50 µl of N-O-bis-(trimethylsilyl)-triflouracetamide (BSTFA). Samples were heated at 50-60°C overnight and reduced under a nitrogen steam the following morning. Internal injection standard (C_{19} FAME) was added to all FAME samples.

Gas chromatographic (GC) analyses were performed with an Agilent Technologies 6890N GC (Palto Alto, California, USA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 mx0.32 mm i.d.), a flame ionization detector (FID), a split/splitless injector and an Agilent Technologies 7683 auto sampler and injector (Phleger et al., 2005). A small number of selected samples representing all tissue types (i.e. ovary, oviduct, mantle and digestive gland) were further analysed using a GC-mass spectrometer (GC-MS) to confirm component identification. GC-MS analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on column injector using Thermoquest Scalibur software (Austin, Texas, USA) and a capillary system comparable to the GC machine (Phleger et al., 2005).

3.3.3 Statistical analyses

All analyses relating to maturation were first considered in terms of seasonal samples. However, if results were similar, all specimens were combined in a single analysis. While all percentage data for analyses was logit transformed, all other raw data was log transformed. Average seasonal differences in size (ML, OW and OVW) were determined using analysis of variance (ANOVA).

In order to determine if there was an energetic trade-off during oogenesis between either the digestive gland or mantle and the ovary, a size adjusted residual analysis was used. Three length-weight type II geometric regression equations using both immature and mature individuals were calculated for ML-MW, ML-DGW and ML-OW. Pearson r correlations (2 tailed) were performed between the resulting unstandardised residuals for ML-OW and ML-DGW, as well as ML-OW and ML-MW. Moreover, to seek evidence of any underlying relationship (or energetic

tradeoff), a principal components analysis was then applied to the weights of the mantle, digestive gland and ovary in conjunction with the total lipid content from each corresponding tissue. Bootstrap 95% confidence ellipses were calculated for the group means of the two maturity groups from 100 samples. These were visualized by plotting the loadings and bootstrap confidence ellipses on the first 2 principal components. Furthermore, to reveal if lipid was being mobilised from either the digestive gland or the mantle to fuel the growth of the ovary, a multivariate analysis of variance (MANOVA) was used to compare the total lipid content of each tissue simultaneously between immature and mature individuals. A canonical variate analysis (CVA) was then used to determine which variables distinguished mature and immature individuals from each other.

The lipid class and fatty acid profiles were compared between maturity stages as well as between the mature ovary and oviduct to determine the importance of these in relation to the embryo and possibly the rhynchoteuthion. Multivariate analysis of variance (MANOVA) was used to simultaneously compare either the average relative levels or the average content of all selected lipid classes and fatty acids between maturity stages (immature and mature) and tissue type (ovary and oviduct). CVA was used to determine the variables that distinguished the groups from each other. Univariate statistics (ANOVA) was then used to compare the relative levels and content of individual lipid classes and fatty acids. An analysis using both univariate and multivariate analyses was also used to compare relative levels or content of lipid classes and fatty acids of the ovary and oviduct according to season. This would assist in ascertaining if seasonal differences in gonad investment (see chapter 2) corresponded to changes in egg quality.

Analyses involving lipid classes were calculated using phospholipid (PL), sterols (ST) and wax ester (WE). Fatty acid analyses were conducted only on those that consistently had percentages greater than 1% of total fatty acids (or 10ng/g).

3.4 Results

3.4.1 General biological information

A total of 42 females were analysed in this study. Twenty-five of these individuals were caught during the autumn months (15 immature, 10 mature) while 17 (8 immature, 9 mature) were caught during the winter months. Mature females caught during the autumn were significantly larger than winter caught individuals ($F=19.13$, $df=1,17$, $p<0.001$) with heavier ovaries ($F=17.31$, $df=1,17$, $p=0.001$) and oviducts ($F=13.36$, $df=1,15$, $p<0.01$). Autumn individuals were caught off southern Tasmania, while the winter sample was a combination of individuals caught off southern and eastern Tasmania.

3.4.2 Energetic tradeoff

Analyses of individuals grouped according to season were similar and therefore the following results represent an overall strategy for females of this species. There was no suggestion of a tradeoff between the ovary and digestive gland, or the ovary and the mantle, as indicated by the lack of any negative correlations between the size-adjusted residuals for these weights. Rather, there was a concomitant increase in ML-MW residuals and ML-OW residuals suggesting that those in better condition also have greater gonad investment ($r=0.40$, $n= 40$, $p<0.05$).

An examination of the trajectories for the weights of the mantle, digestive gland and ovary, and their corresponding lipid content yielded two principal components that accounted for 53 and 19 percent of variation in the data, respectively. The first component appeared to be size related. Mature females separated from immature females clearly along this component, signifying a synchronous increase in MW, DGW, OW and ovary lipid content. Nevertheless, the second component was of particular interest as it was a contrast of increasing mantle lipid content versus decreasing digestive gland lipid content. An examination of the loadings and bootstrap confidence ellipses along this component was suggestive of a limited tradeoff. As females matured there was a concurrent increase in mantle and ovary lipid content, but a corresponding decrease in digestive gland lipid content (Fig. 3.1).

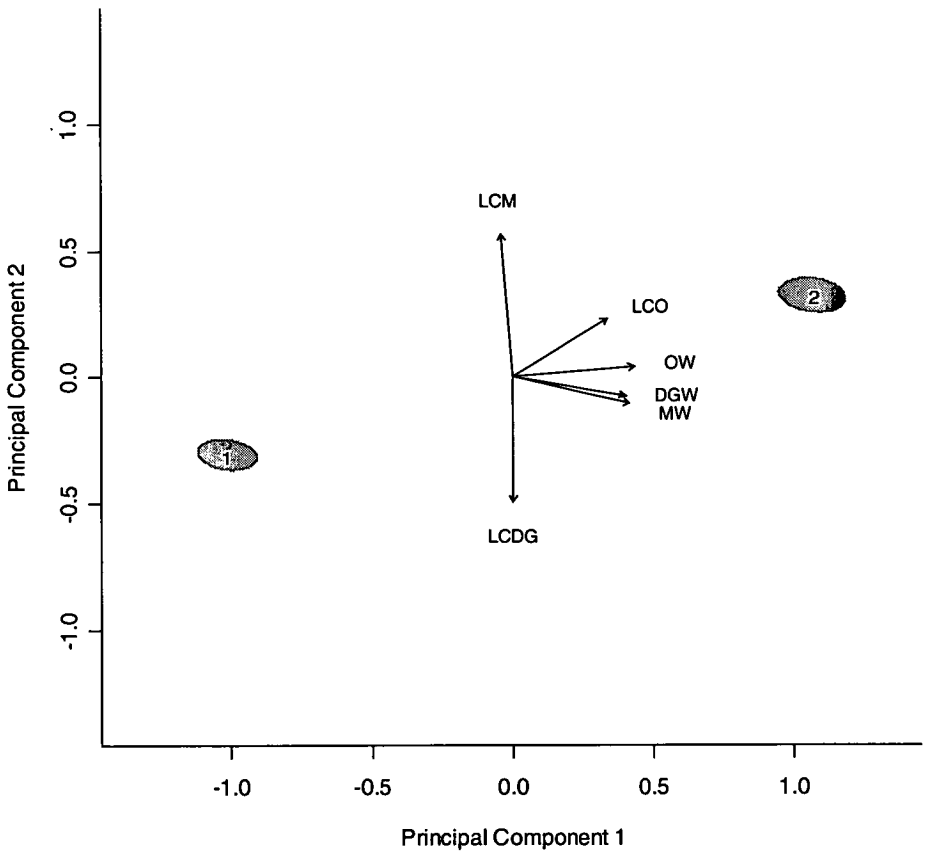

Figure 3.1: *Todarodes filippovae.* Plot showing the loadings and bootstrap 95% confidence ellipses for the group means of the two maturity groups of females (1= immature, 2= mature) on the first two principle components based on the lipid content (mg/g) and the weight of the corresponding tissue. LCM=lipid content mantle, LCO=lipid content ovary, OW=ovary weight, DGW= digestive gland weight, MW=mantle weight, LCDG=lipid content digestive gland.

Mature females were significantly distinguished from immature females when the total lipid content (mg/g wet mass) of the digestive gland, mantle and ovary were compared concurrently (Wilks Lambda = 0.354, df=3,34, $p<0.001$). CVA revealed that this was due solely to the lipid content in the ovary. Mature females had an increasing amount of lipid in the ovary compared to immature females. Similarly, univariate analyses also confirmed that on an individual basis the ovary had significantly higher amounts of lipid (mg/g wet mass) in mature individuals ($F=67.86$, df=1,38, $p<0.001$). However, there were no observed differences between mature and immature individuals for the lipid content of the digestive gland or the mantle (Fig. 3.2).

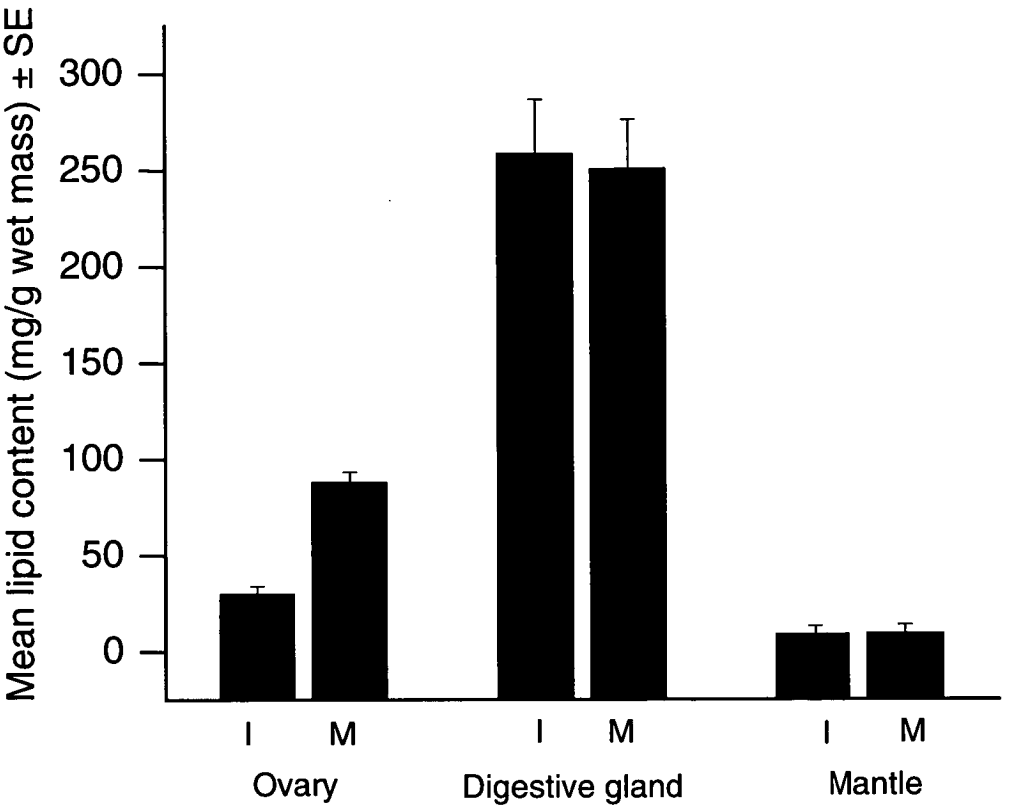

Figure 3.2: *Todarodes filippovae.* Mean lipid content (mg/g wet mass) of the ovary, digestive gland, and mantle for immature (I) and mature (M) females. Some lipid content for digestive gland and mantle are taken from Pethybridge (2004), SE=standard error.

3.4.3 Lipid profile of oocytes and eggs

Lipid content (mg/g wet mass) in the ovary and the oviduct, although relatively uniform, was greater than that of the mantle, but much lower than that of the digestive gland. Lipid in the ovary and oviduct was comprised of 4 main classes. Phospholipid (PL) constituted the largest lipid fraction (up to 90%). The next most abundant lipid class was ST (7-12%), which almost exclusively was cholesterol. Both WE and FFA had similar minor relative levels (1-2%). Low FFA levels in the ovary and oviduct, as well as the digestive gland and mantle, indicated good sample integrity with low lipolytic and enzyme activity. The overall lipid class compositions of the mantle were similar to the ovary and oviduct, with the only difference being the addition of a small percentage of TAG. In contrast, the digestive gland had large relative levels of TAG and comparatively minor portions of PL (Table 3.1).

Table 3.1: *Todarodes filippovae.* Percent lipid class composition (of total lipids) and content (% wet mass) of mature oviducts and ovaries from autumn and winter; and of the ovary, digestive gland and mantle for immature and mature females. Values presented as mean ± standard deviation. WE, wax ester; DAG, diacylglycerol; TAG, triacylglycerol; FFA, free fatty acid; ST, sterol; PL, phospholipid; HC, hydrocarbon. n = sample size, n in parentheses denotes change in sample size for total lipids. Some lipid class and lipid content data for digestive gland and mantle is from Pethybridge (2004).

Lipid class	Oviduct		Ovary (mature)		Ovary		Digestive gland		Mantle	
	Autumn	Winter	Autumn	Winter	Immature	Mature	Immature	Mature	Immature	Mature
n	10	7	10	9	23	19	20 (21)	18 (19)	21 (19)	19
WE	2.0 ± 0.6	3.3 ± 0.6	2.3 ± 0.6	2.2 ± 0.9	1.4 ± 1.0	2.3 ± 0.7	3.6 ± 2.2	4.1 ± 2.3	0.1 ± 0.2	0.1 ± 0.4
DAGE	0.0	0.0	0.0	0.0	0.0	0.0	2.4 ± 2.2	2.3 ± 1.6	0.0	0.0
TAG	0.0	0.0	0.0	0.0	0.0	0.0	77.9 ± 11.6	81.0 ± 6.1	1.1 ± 2.9	0.8 ± 1.3
FFA	0.2 ± 0.2	0.3 ± 0.5	0.8 ± 0.4	0.6 ± 0.5	2.3 ± 1.7	0.8 ± 0.5	5.3 ± 4.5	5.0 ± 2.5	0.8 ± 0.4	0.9 ± 0.7
ST	8.2 ± 1.3	9.1 ± 1.9	7.7 ± 0.7	7.9 ± 1.6	11.5 ± 4.4	7.6 ± 0.7	1.5 ± 1.0	0.8 ± 0.6	10.8 ± 3.3	10.3 ± 2.4
PL	89.5 ± 1.2	87.3 ± 2.1	89.2 ± 1.1	89.3 ± 1.3	84.9 ± 5.0	89.3 ± 1.1	5.0 ± 4.8	3.6 ± 3.3	87.2 ± 4.7	87.9 ± 3.7
HC	0.0	0.0	0.0	0.0	0.0	0.0	1.2 ± 2.0	0.6 ± 1.0	0.0	0.0
Lipid content[a]	6.6 ± 3.9	8.0 ± 2.2	8.8 ± 7.0	7.7 ± 3.4	2.9 ± 1.9	8.8 ± 2.3	25.9 ± 12.9	25.1 ± 11.3	0.9 ± 0.5	0.9 ± 0.5

[a]note: for lipid content 1% (wet mass) = 10 mg/g (wet mass)

The fatty acid profile of the ovary was dominated by saturated fatty acids (SFA), which comprised around 40% of total fatty acids, for both immature and mature individuals. The main SFA were 16:0 and 18:0. Similarly, polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) were consistent between maturity groups and accounted for approximately 35% and 25% of total fatty acids respectively. PUFA was composed predominantly of the essential fatty acids, docosahexaenoic acid (DHA: 22:6(n-3)) and eicosapentaenoic acid (EPA; 20:5(n-3)), while MUFA was dominated by 20:1(n-9)c. The fatty acid 18:1(n-9)c also accounted for a major proportion of MUFA (Table 3.2).

3.4.4 Differences due to maturation

There were a number of important differences between the lipid class and fatty acid profiles of the oocyte in the ovary when assessed in relation to maturation. Separate seasonal analyses were not calculated, as maturational differences were consistent when compared within seasons. Differences in both the lipid classes as well as the fatty acids are firstly reported in terms of the relative levels (%) then the absolute amount (mg/g or ng/g). Furthermore, significant differences in the average relative levels and amounts of the lipid classes are initially reported using univariate statistics, followed by the results of multivariate analysis that considered simultaneously all the lipid classes and fatty acids analysed.

Table 3.2: *Todarodes filippovae.* Percent composition (means ± standard deviation) and content (ng/g) of total fatty acids of the ovary of immature (stage 2 and 3) and mature (stage 5) females. Number in parentheses denotes sample size (n).

	Immature (22)	Mature (19)
14:0	1.0 ± 0.5	0.8 ± 0.2
16:0	26.9 ± 4.5	27.7 ± 4.7
17:0	1.5 ± 0.3	1.3 ± 0.3
18:0	10.2 ± 2.0	9.3 ± 1.8
18:1(n-7)c	1.4 ± 0.3	1.7 ± 0.3
18:1(n-9)c[a]	4.3 ± 1.6	7.5 ± 1.3
20:1(n-9)c	14.7 ± 2.4	12.4 ± 1.9
22:1(n-9)c + 16:0 GED[b]	1.9 ± 0.8	0.9 ± 0.2
20:2(n-6)	0.7 ± 0.2	0.8 ± 0.4
20:3(n-6) + 20:2 NMID[b]	0.3 ± 0.2	0.8 ± 0.2
20:4(n-3) + 20:2 NMID[b]	0.3 ± 0.1	0.5 ± 0.2
20:4(n-6) AA	1.5 ± 0.8	0.7 ± 0.2
20:5(n-3) EPA	11.6 ± 4.6	8.8 ± 2.6
22:2 NMI	0.6 ± 0.2	0.8 ± 0.3
22:5(n-3) DPA	0.7 ± 0.3	0.6 ± 0.2
22:6(n-3) DHA	17.9 ± 7.0	21.0 ± 7.3
C23 PUFA	0.4 ± 0.2	0.5 ± 0.2
Others	3.9	3.6
Sum SFA	41.1 ± 6.9	40.4 ± 7.0
Sum MUFA	24.1 ± 3.7	24.6 ± 3.3
Sum PUFA	34.5 ±10.2	34.8 ± 9.9
Sum (n-3)	30.5 ±10.1	30.9 ± 10.1
Sum (n-6)	3.0 ± 0.8	2.7 ± 0.3
Ratio (n-3)/(n-6)	10.4 ± 3.8	11.7 ± 4.0
Ratio EPA/AA	8.8 ± 2.5	12.8 ± 2.6
Ratio DHA/EPA	1.6 ± 0.6	2.3 ± 0.2
Total FA (ng/g)	564.7 ± 387.4	1455.7 ± 805.3

[a]18:3(n-3) is coeluting with 18:1(n-9)c; [b]where two FA are shown together, the latter minor FA coelutes with the former; GED, glyceryl ether diol; NMID nonmethylene interrupted diunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; other includes components present at <0.5%: 14:1(n-5)c, 15:0, i15:0, a15:0, i16:0, 16:1(n-5)c, 16:1(n-7)c, 16: 1(n-7)t, 16:1(n-9)c, i17:0, a17:0, 17:1(n-6), i18:0 + 18:4(n-3), 18:1(n-5)c, 18:1(n-7)t, 18:2(n-6), 19:1, 20:0, 20:1(n-7)c, 21:5(n-3), 22:0, 22:1(n-7), 22:1(n-11/13), 22:4(n-6), 22:5(n-6), C22 PUFA.

3.4.4.1 Lipid classes

Based on average values, oocytes of mature individuals had significantly higher relative levels of PL ($F=14.48$, $df=1,40$, $p<0.001$) and WE ($F=11.87$, $df=1,40$, $p<0.01$), but lower levels of ST ($F=13.65$, $df=1,40$, $p<0.01$). Multivariate analysis of variance (MANOVA) also revealed evidence of a significant difference in the lipid class composition between the oocytes of mature and immature females (Wilks Lambda = 0.68, $df=3,38$, $p<0.01$). Although the average relative level of ST was lower in mature ovaries, a canonical variate distinguished mature individuals from immature individuals based on all three classes increasing together (Fig. 3.3a).

Although the average relative levels of ST were significantly less in mature oocytes than immature oocytes, this was not the case for the absolute amounts. Mature females had significantly greater amounts (mg/g) of all three classes in the ovary, that is, PL ($F=70.38$, $df=1,40$, $p<0.001$), ST ($F=65.92$, $df=1,40$, $p<0.001$) and WE ($F=42.64$, $df=1,40$, $p<0.001$). Whereas MANOVA also highlighted a significant difference between mature and immature females for all three lipid classes when compared simultaneously (Wilks Lambda=0.34, $df=3,38$, $p<0.001$), female ovaries were distinguished based on a canonical variate of increasing ST and PL. Mature females continued to accumulate greater amounts of ST and PL relative to the pattern observed between the groups. Although WE also increased in mature females, it was not determined to be an important discriminating variable as it was for distinguishing differences based on the relative levels of classes (Fig 3.3b).

3.4.4.2 Fatty Acids

There were 8 fatty acids that consistently had greater than 1% (or 10ng/g) of total fatty acids that were statistically treated in all fatty acid analyses. These were 16:0, 17:0, 18:0, 18:1(n-7)c, 18:1(n-9)c, 20:1(n-9)c, EPA and DHA. Across seasonal samples these 8 fatty acids accounted for 90% of the total fatty acids in the mature ovary and oviduct. Univariate statistics using these fatty acids revealed that mature females had significantly lower relative levels of EPA ($F=4.89$, $df=1,39$, $p<0.05$) and 20:1(n-9)c ($F=10.65$, $df=1,39$, $p<0.01$), and higher relative levels of 18:1(n-9)c ($F=42.44$, $df=1,39$, $p<0.001$) and 18:1(n-7)c ($F=14.34$, $df=1,39$, $p<0.01$). Although the average levels of DHA in the oocytes did not differ due to maturation, the

essential fatty acid arachidonic acid (AA: 20:4(n-6)) was lower in the ovary of mature (0.7 ± 0.2%) than immature (1.5 ± 0.8%) individuals. MANOVA also showed evidence of a significant compositional difference between immature and mature ovaries when all 8 fatty acids were compared concurrently (Wilks Lambda = 0.27, df=8,32, $p<0.001$). Canonical variate analysis differentiated the oocytes of the females based on a contrast between increasing DHA, 16:0 and 20:1(n-9)c; and decreasing 18:1(n-9)c. Relative to this pattern observed between the two groups, mature females had increasing percentages of 18:1(n-9)c and decreasing percentages of DHA, 16:0 and 20:1(n-9)c. It is evident that although the average levels of DHA and 16:0 were not different between mature and immature individuals, they were showing a decreasing level of accumulation in the mature ovary (Fig 3.3c).

The average absolute amount (ng/g wet mass) of each individual fatty acid in the mature ovary was significantly higher than that recorded for immature ovaries ($F>12.25$, df=1,39, $p<0.01$, Table 2). In contrast, when the interrelationships between the content of each fatty acid was considered, the fatty acids were not all increasing together even though the absolute amounts were still higher in the mature ovaries. CVA determined that the significant maturational difference highlighted by MANOVA (Wilks Lambda=0.32, df=8,32, $p<0.001$) was due to a contrast predominantly between increasing 18:1(n-9)c, 18:1(n-7)c and EPA; and decreasing 20:1(n-9c) and DHA. In accordance with the observed pattern between the oocytes in the ovaries of mature and immature females, mature females had increasing amounts of 18:1(n-9)c, 18:1(n-7)c and EPA, and decreasing amounts of 20:1(n-9c) and DHA (Fig 3.3d).

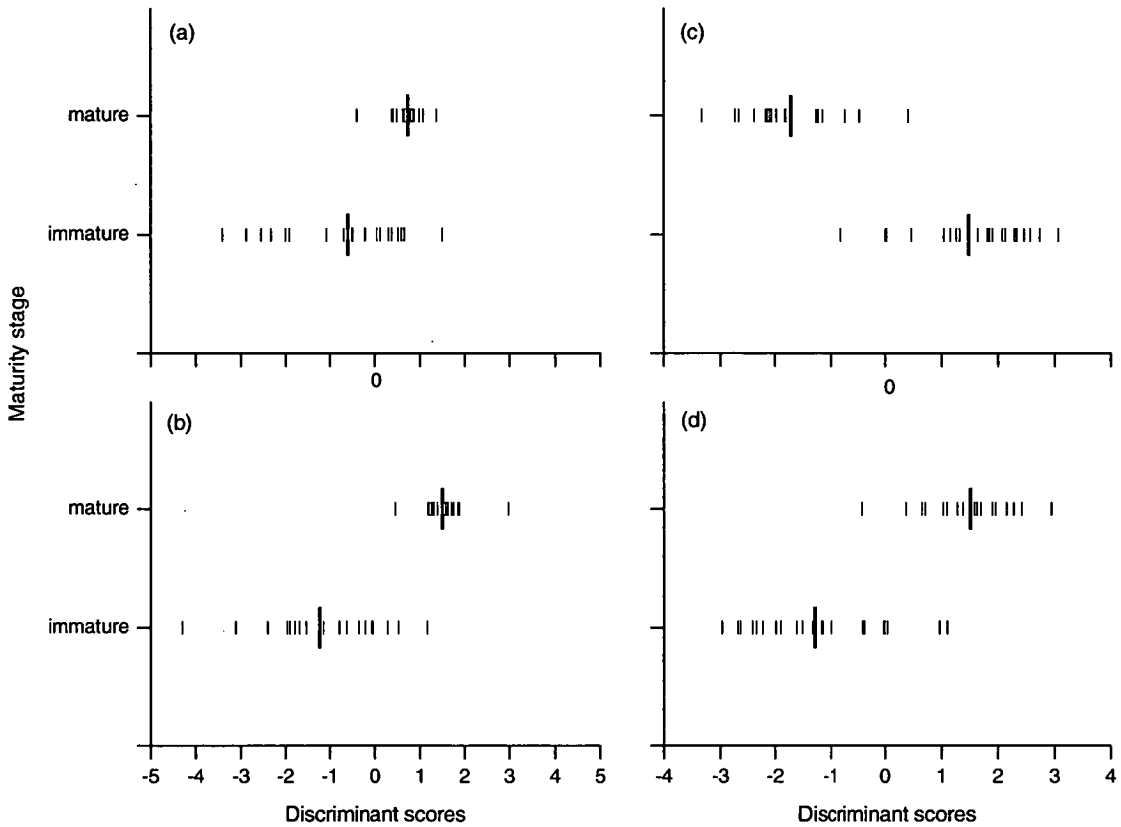

Figure 3.3: *Todarodes filippovae.* Discriminant scores from canonical variate analyses on the (a) relative levels of lipid class, (b) lipid class content, (c) relative levels of fatty acids and (d) fatty acid content for immature and mature females. Lipid class analyses included phospholipid, sterol and wax ester. Fatty acid analyses included 16:0, 17:0, 18:0, 18:1(n-7)c, 18:1(n-9)c, 20:1(n-9)c, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). The long dash in each maturity group represents the group centroid.

Mature females likewise had higher average amounts of SFA ($F=24.1$, $df=1,39$, $P<0.001$), MUFA ($F=26.33$, $df=1,39$, $p<0.001$) and PUFA ($F=18.96$, $df=1,39$, $P<0.001$) in the ovary than immature females (Fig. 3.4). A significant difference between mature and immature females was found when comparing all the sums simultaneously (Wilks Lambda=0.58, $df=3,37$, $p<0.001$). When within group correlations were taken into account, CVA distinguished the ovary of mature females from immature females by the increasing sums of PUFA and decreasing sums of SFA. Furthermore, although the n-3/n-6 ratio didn't change due to maturation, the

EPA/AA (F=21.53, df=1,39, p<0.001) and DHA/EPA (F=21.93, df=1,39, p<0.001) ratios were higher in mature ovaries than immature ovaries.

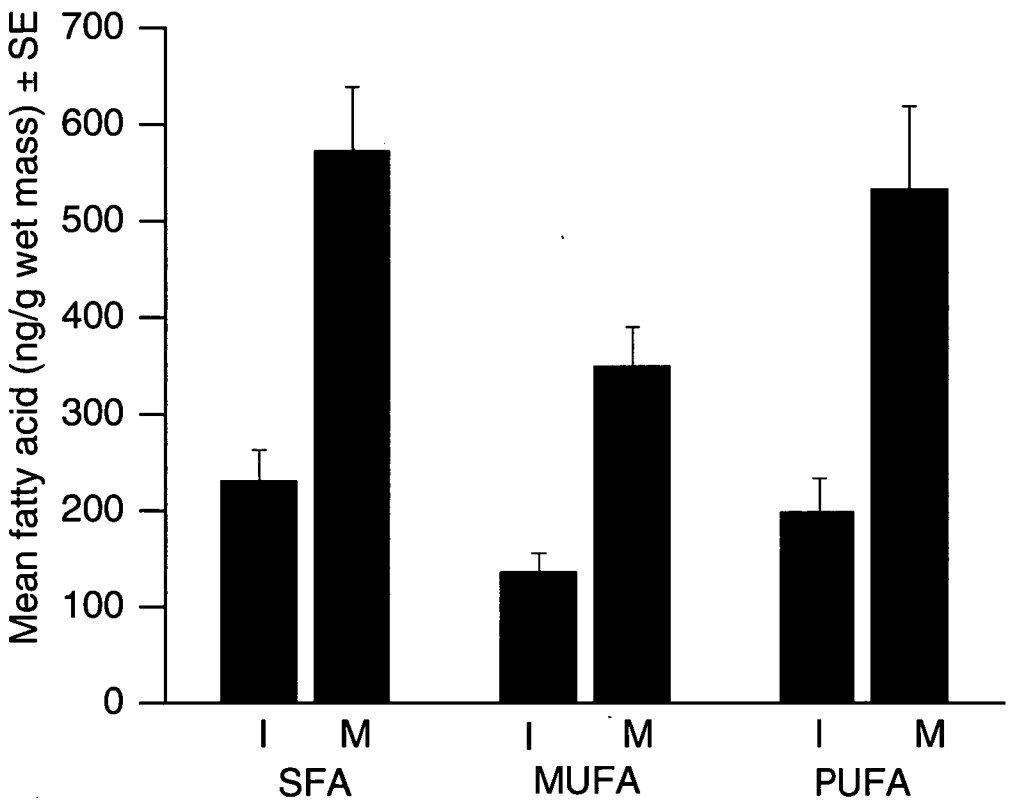

Figure 3.4: *Todarodes filippovae.* Mean fatty acid content (ng/g wet mass) grouped according to saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of the ovary for immature (I) and mature (M) females, SE=standard error.

3.4.5 Differences between mature oocyte and ovulated egg

Differences between the oocyte in the ovary and ovulated eggs in the oviduct were minimal and not consistent between seasons. Since some differences were found within the seasons, analyses were computed for the seasons separately.

3.4.5.1 Lipid class

There was no significant difference in the lipid content (mg/g wet mass) between the oocytes in the ovary (consisting of varying stages of development) and the mature eggs of the oviduct. The autumn sample was nearing significance (p=0.07), and an

examination of the means showed that oviduct eggs tended to have lower lipid content than the ovarian oocytes. Univariate analysis also revealed no significant differences in any of the relative levels (% wet mass) of lipid classes between the ovary and oviduct in autumn. However, winter oviducts had significantly higher average relative levels of WE ($F=7.69$, $df=1,14$, $p<0.05$) and ST ($F=6.09$, $df=1,14$, $p<0.05$), but lower levels of PL ($F=6.65$, $df=1,14$, $p<0.05$) compared to the winter ovary. When comparing the classes between the ovary and the oviduct concurrently for each season, MANOVA yielded significant differences for both autumn (Wilks Lambda=0.40, $df=3,16$, $p<0.01$) and winter females (Wilks Lambda=0.471, $df=3,12$, $p<0.05$). In relation to the pattern discriminating between the tissues in each season, the oviduct had higher relative levels of all lipid classes, but in particular ST (Fig 3.5). This suggests that although the oviduct did not always have greater amounts of each class than the ovary, they were continuing to increase simultaneously.

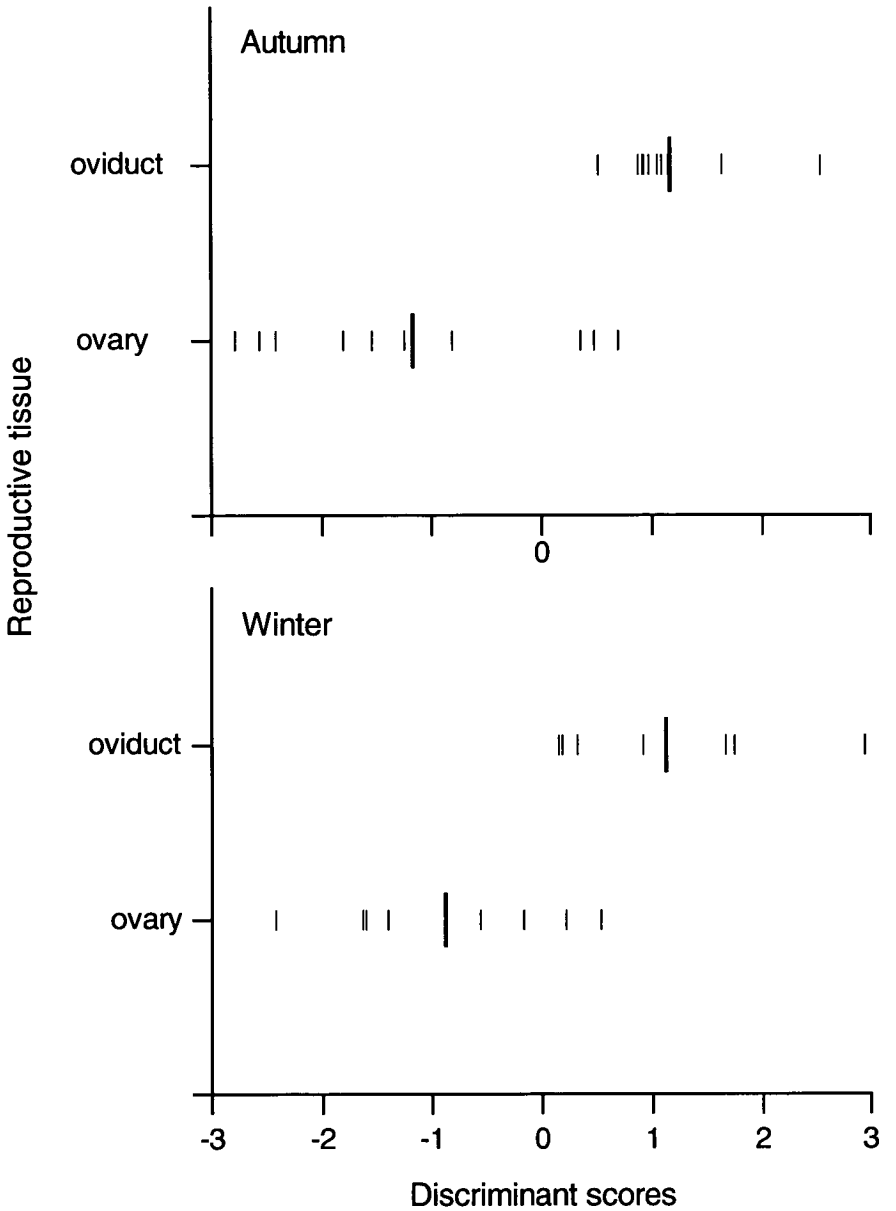

Figure 3.5: *Todarodes filippovae.* Discriminant scores from canonical variate analyses on the relative levels of lipid classes in the ovary and oviduct of mature females caught in autumn and winter. Lipid class analyses included phospholipid, sterol and wax ester. The long dash in each reproductive tissue group represents the group centroid.

No significant univariate differences between the reproductive tissues were found for autumn or winter in the amount of lipid (mg/g wet mass) for each class. However, based on MANOVA the oocytes also differed from the eggs in the winter sample (Wilks Lambda=0.506, df=3,12, $p<0.05$). The oviduct was separated from the ovary

based on a contrast between decreasing PL and increasing WE and ST. Although the oviduct did not have greater absolute amounts of each class, when compared simultaneously, ST and WE increased together, while the accumulation of PL decreased.

3.4.5.2 Fatty acids

There were no significant differences in the fatty acid composition or the absolute amount of the 8 fatty acids analysed between the mature ovary and oviduct in either season. However, when comparing the amount of individual fatty acids in multivariate space, a significant difference between these two reproductive organs was evident in the autumn group (Wilks Lambda=0.31, df=8,11, $p<0.05$). The organs were predominantly distinguished by a contrast between two essential fatty acids, that is, increasing EPA and decreasing DHA. Compared to the ovary, the oviduct continued to accumulate DHA but the amount of EPA accumulating was decreasing.

No observed differences between the oocytes in the ovary or the mature eggs in the oviduct were due to variation in the total amounts of SFA, MUFA and PUFA. In terms of the DHA/AA, EPA/AA and n-3/n-6 ratios, again there was no variation observed.

3.4.6 Seasonal differences

There were few seasonal differences in the ovary or in the oviduct. Most observed differences were in the oviduct and were evident when comparing within multivariate space due to the interrelationships between the classes and fatty acids.

3.4.6.1 Lipid class

Lipid content in the oviduct or ovary did not vary significantly between seasons. A single individual consistently appeared as a suspected outlier across a number of analyses. In all but this analysis, deleting the observation did not alter the conclusions and the observation was retained. Autumn females were found to have

significantly higher lipid content in the ovary than winter females when the outlier was removed from the winter sample ($F=7.482$, $df = 1,16$, $p<0.05$) (Fig. 3.6).

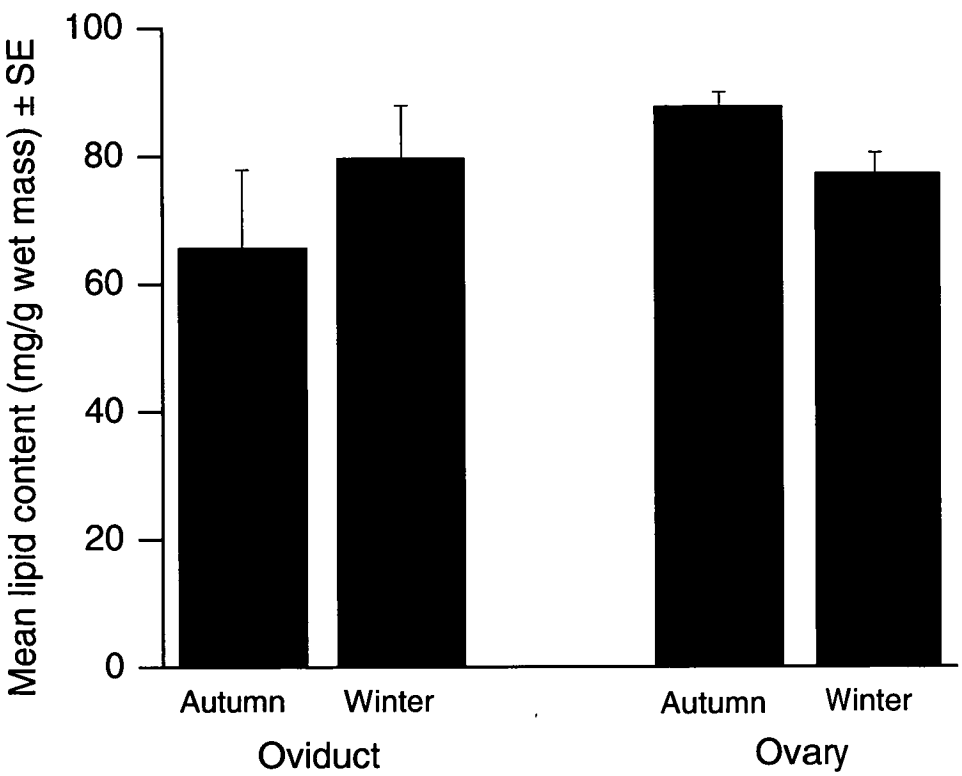

Figure 3.6: *Todarodes filippovae.* Mean lipid content in the mature oviduct and ovary for autumn and winter females, SE=standard error.

Both univariate and multivariate statistics failed to reveal any significant differences in the mature ovary when comparing seasonal differences in the relative levels or content of the lipid classes. However, the relative levels of WE were higher in the oviducts of winter females than autumn females ($F=7.75$, $df=1,15$, $p<0.05$). Conversely, relative levels of PL in the oviducts were lower in winter than in autumn caught females ($F=8.15$,$df=1,15$, $p<0.05$).

Furthermore, there was a significant seasonal difference in the relative levels of lipid classes between the oviducts when all 3 lipid classes (PL, ST, WE) were compared concomitantly (Wilks Lamba = 0.411, $df=3,13$, $p<0.01$). A canonical variate discriminated between autumn and winter females based on PL and ST levels. In

relation to the pattern separating the seasons, autumn oviducts had increasing relative levels of PL and ST (Fig. 3.7a). However, an examination of the residuals revealed an extreme WE outlier. Subsequent to this outlier being removed, the importance of ST as a discriminating variable between seasons was reduced, while the importance of WE was strengthened. Autumn oviducts were still separated from winter oviducts by increasing PL, and to a lesser extent percentage of ST, but also decreasing relative levels of WE.

There were no significant seasonal variations between the amounts of lipid for each class using univariate analysis. Conversely, in multivariate space, a significant seasonal variation in the amount of WE, PL and ST in the oviduct was evident (Wilks Lambda = 0.49, df=3,13, $p<0.05$). A canonical variate determined that the seasonal variation was based on a contrast between increasing WE and ST and decreasing PL. In relation to this contrasting pattern between the seasons, the accumulation of PL in the autumn oviducts was continuing to increase in comparison to winter, while there was a decreasing pattern observed for WE and ST (Fig 3.7b).

3.4.6.2 Fatty acids

The fatty acid profile based on seasonal differences was relatively conserved. There were no significant differences in the mature ovary or oviduct when comparing the average relative levels of the fatty acids. However, a significant seasonal difference in the percentage of all 8 fatty acids in the oviduct was apparent (Wilks Lambda=0.192, df=8,8, $p<0.05$). Autumn samples were distinguished from winter samples by a higher level of 17:0 and lower levels of DHA, 16:0, EPA, 18:1(n-9)c and to lesser extent 18:0 and 18:1(n-7) based on the relative pattern between the seasons produced by CVA (Fig. 3.7c). Although there were no differences in average levels of the fatty acids, in autumn some levels were continuing to increase while others were decreasing in comparison to winter. It is particularly noteworthy that levels of DHA and EPA were increasing in autumn oviducts.

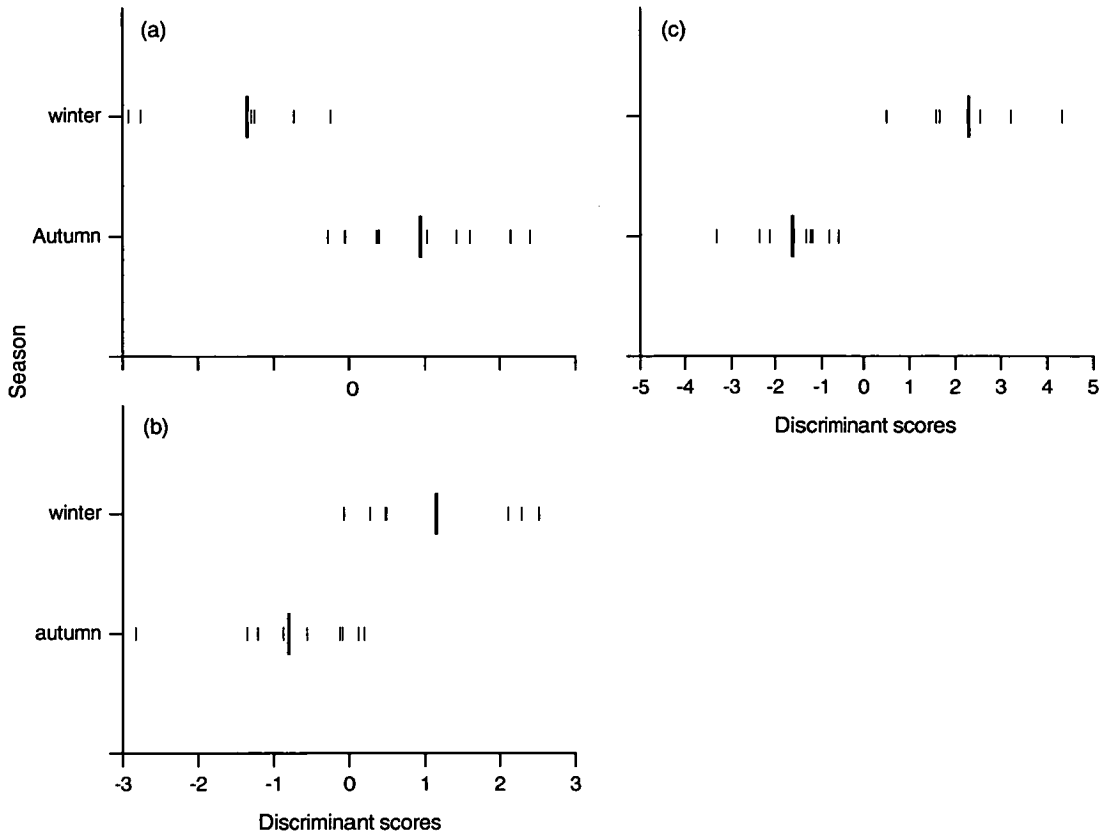

Figure 3.7: *Todarodes filippovae.* Discriminant scores from canonical variate analyses on the (a) relative levels of lipid classes, (b) lipid class content and (c) relative levels of fatty acids in the oviduct of mature females caught in autumn and winter. Lipid class analyses included phospholipid, sterol and wax ester. Fatty acid analysis included 16:0, 17:0, 18:0, 18:1(n-7)c, 18:1(n-9)c, 20:1(n-9)c, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). The long dash in seasonal group represents the group centroid.

The amount of each FA (ng/g) was fairly uniform between autumn and winter for both the ovary and oviduct, and hence no significant univariate or multivariate differences were found. Furthermore, the fatty acid profile of the oviduct mirrored that of the mature ovary. The fatty acid profile included 40% SFA which was dominated by 16:0 and 18:0, MUFA which was dominated by 20:1(n-9)c, while PUFA, which was composed mainly of DHA and EPA accounted for approximately 35% of the total fatty acid (Table 3.3).

There was no observed variation between the sums of saturated, monounsaturated or polyunsaturated fatty acids in response to season. Similarly, there were no seasonal differences between the mean ratios of n-3/n-6, DHA/AA or EPA/AA.

Table 3.3: *Todarodes filippovae.* Percent composition (mean ± standard deviation) and content (ng/g) of total fatty acids in the ovary and oviduct of mature (stage 5) females from autumn and winter. Number in parentheses denotes sample size (n).

	Ovary		Oviduct	
	Autumn (10)	Winter (9)	Autumn (10)	Winter (7)
14:0	0.8 ± 0.2	0.9 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
16:0	27.5 ± 5.2	27.9 ± 4.5	27.6 ± 4.8	27.5 ± 1.9
17:0	1.4 ± 0.3	1.2 ± 0.3	1.4 ± 0.3	1.2 ± 0.2
18:0	9.5 ± 2.1	9.1 ± 1.6	9.9 ± 1.2	9.4 ± 1.0
i18:0+18:4(n-3)[a]	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
18:1(n-7)c	1.7 ± 0.3	1.7 ± 0.3	1.6 ± 0.2	1.7 ± 0.3
18:1(n-9)c[b]	7.5 ± 1.0	7.5 ± 1.6	7.8 ± 1.2	8.2 ± 0.9
20:1(n-9)c	13.0 ± 2.1	11.8 ± 1.6	13.0 ± 1.4	12.1 ± 1.1
22:1(n-9)c+16:0 GED[a]	1.0 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.6 ± 0.2
20:2(n-6)	0.7 ± 0.2	0.9 ± 0.4	0.6 ± 0.2	0.6 ± 0.2
20:3(n-6)+20:2 NMID[a]	0.9 ± 0.2	0.7 ± 0.1	1.0 ± 0.2	0.9 ± 0.1
20:4(n-3)+20:2 NMID[a]	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
20:4(n-6) AA	0.7 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0.6 ± 0.1
20:5(n-3) EPA	8.4 ± 2.9	9.3 ± 2.4	8.0 ± 2.2	8.7 ± 1.2
22:2 NMI	0.9 ± 0.4	0.7 ± 0.2	0.9 ± 0.3	0.8 ± 0.1
22:5(n-3) DPA	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.1
22:6(n-3) DHA	20.1 ± 7.8	21.9 ± 7.1	20.8 ± 7.0	21.7 ± 3.4
C23 PUFA	0.5 ± 0.3	0.5 ± 0.2	0.4 ± 0.2	0.3 ± 0.2
Others	3.6	2.7	3.8	3.0
Sum SFA	40.6 ± 7.7	40.1 ± 6.5	40.8 ± 6.5	40.1 ± 2.9
Sum MUFA	25.3 ± 3.4	23.7 ± 3.2	25.2 ± 2.9	24.5 ± 1.7
Sum PUFA	33.8 ± 10.7	36.0 ± 9.5	33.8 ± 9.0	35.2 ± 4.4
Sum (n-3)	29.8 ± 10.9	32.2 ± 9.7	30.0 ± 9.3	31.5 ± 4.7
Sum (n-6)	2.7 ± 0.3	2.7 ± 0.3	2.6 ± 0.3	2.7 ± 0.3
Ratio (n-3)/(n-6)	11.1	12.3	11.8	11.9
Ratio EPA/AA	13.1	12.4	15.0	14.5
Ratio DHA/EPA	2.3	2.3	2.6	2.5
Total FA ng/g	1608.0 ± 691	1286.0 ± 927	1097.0 ± 660	1561.0 ± 526

[a] Where two FA are shown together, the latter minor FA coelutes with the former FA; [b]18:3(n-3) is coeluting with 18:1(n-9)c; GED, glyceryl ether diol, NMID nonmethylene interrupted diunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; other includes components present at <0.5%: 15:0, i15:0, a15:0, i16:0, 16:1(n-5)c, 16:1(n-7)c, 16: 1(n-7)t, 16:1(n-9)c, i17:0, a17:0, 17:1(n-6), 18:1(n-5)c, 18:1(n-7)t, 18:2(n-6), 19:1, 20:0, 20:1(n-7)c, 21:5(n-3), 22:0, 22:1(n-7), 22:1(n-11/13), 22:4(n-6), 22:5(n-6), C22 PUFA.

3.5 Discussion

The marked twofold increase of lipid content in the ovary with maturation alludes to the importance of lipid in embryonic development and potentially for hatchlings of *Todarodes filippovae*. This is despite the predominance of a protein-based metabolism (Lee, 1994) in conjunction with a constrained ability to digest lipids (O'Dor et al., 1984) for cephalopods in general. Overall, there was limited evidence of lipid being used as an energy reserve for reproduction. Similarly, lipid in the ovary or oviduct did not figure as a principal reserve for the development of the embryo or subsequent rhynchoteuthion. Rather, lipid appears conserved for a specific purpose. A structural role for lipid in the reproductive organs was evidenced by the presence of predominantly polar lipids and relatively limited amounts of neutral lipid (e.g. triglyceride). The significant role of ovarian lipid in *T. filippovae* was further highlighted by the dominance of essential fatty acids in the PUFA fraction. Moreover, observed seasonal differences in lipid content or classes might largely reflect reproductive plasticity in response to the environment.

3.5.1 Energetic tradeoff

Compared to the mantle and the reproductive organs, lipid content in the digestive gland of female individuals of *T. filippovae* was extremely elevated. Relatively high lipid content have also been found in the digestive gland of sub-Antarctic and Antarctic squid species *Moroteuthis robsoni* (35%), *M. ingens* (42%) *Psychroteuthis glacialis* (32%), *Mesonychoteuthis hamiltoni* (32%), and *Gonatus antarcticus* (54%) (Phillips et al., 2001, 2002; Phillips, 2003). Lipid has a very high energy value (Parrish et al., 2005) and therefore it would appear incongruous that an animal with an organ rich in lipid would not catabolise this potentially abundant reservoir of energy. Nevertheless, in this study there was little evidence for mobilisation of energy from the digestive gland for reproduction, particularly at the whole animal level. Neither was there any evidence that energy was being mobilised from the mantle for reproduction. On the contrary, mantle condition increased with maturation. Correspondingly, an analysis of the process of energy allocation in response to reproduction in the ommastrephid *Nototadarus gouldi* also failed to reveal any tradeoffs with either the mantle or digestive gland at the whole animal level (McGrath & Jackson, 2002).

Despite the lack of evidence to suggest an energetic tradeoff at the whole animal level, principal component analysis indicated a small tradeoff between the digestive gland and mantle at the biochemical level. It appeared that, as ovary lipid content increased significantly with maturation, there was a simultaneous increase in mantle lipid content and a decrease in digestive gland lipid content. Additionally, the presence of large amounts of TAG, often referred to as a short-term energy reserve, in the digestive gland of female *T. filippovae* implicated this organ as a potential lipid reserve for reproduction. However, it is unlikely that lipid was being mobilised as a significant energy reserve for maturation since the mantle and digestive gland lipid content did not vary between immature and mature individuals. Rosa et al. (2005) reported that biochemical constituents of the digestive gland, mantle and gonad of the ommastrephids *Illex coindettii* and *Todaropsis eblanae* varied independently of one another, also suggestive of a lack of evidence for an energetic tradeoff. Moreover, they found an increasing trend with maturation in both digestive gland and ovary lipid content of the two ommastrephid species, although total lipid content in the mantle did not vary significantly. Furthermore, given the limited digestibility and slow assimilation of lipid in cephalopods (O'Dor et al., 1984; O'Dor & Wells, 1987), the use of lipid in the digestive gland as a substantial storage substrate is improbable. Semmens (1998) found that excess dietary lipid in two tropical loliginids was being bundled in brown vacuoles in readiness for excretion rather than being stored. Therefore, a likely explanation for the decrease in digestive gland lipid with maturation in female *T. filippovae* is that as the requirements for ovary lipid significantly increased, there was a small decrease in the amount of superfluous dietary lipid being deposited in the digestive gland.

In some squid, digestive gland lipid has been suggested as an aid in buoyancy. For cephalopods this would mean a tradeoff between locomotion and the associated cost of maintaining their position in the water column (O'Dor et al., 2002; Seibel et al., 2004). The added drag and increased volume in the mantle cavity (Seibel et al., 2004) would not likely be compatible with a large muscular visual predator (O'Dor et al., 2002) such as *T. filippovae* inhabiting pelagic waters. Although lipids allow extensive vertical movement (O'Dor, 2002), it is suggested that negatively buoyant squid that migrate horizontally and vertically may save energy by using climb and

glide swimming (O'Dor & Dawe, 1998). High lipid content in the digestive gland is more likely to act as a significant buoyancy mechanism for slow-moving squid or those living permanently at depth.

Todarodes filippovae is likely to predominantly adhere to the direct acquisition of food model (see chapter 2) to fuel oogenesis rather than through the mobilisation of stored nutrients. A similar model has been proposed for *Photololigo* sp (Moltshaniwskyj & Semmens, 2000) *Octopus vulgaris*, *O. defilippi* (Rosa et al., 2004) and other ommastrephids including *Stenoteuthis oualensis* (Harman et al., 1989), *N. gouldi* (McGrath & Jackson, 2002), *I. coindettii* and *T. eblanae* (Rosa et al., 2005). The possession of a voracious and opportunistic predatory diet in association with high metabolism and conversion rates enables these cephalopods to grow in preference to storing energy (O'Dor & Webber, 1986). During starvation periods, *O. vulgaris* and *Sepia officinalis* extensively used protein as an energetic fuel (O'Dor et al., 1984; Castro et al., 1992). Furthermore O'Dor et al (1984) suggest that protein was the most conserved nutrient during starvation and that it is non-limiting. Therefore, it is likely that when food resources are insufficient to meet the higher energy requirements of reproduction, individual energy needs may be best met by the catabolism of mantle protein, the premium energy reserve for cephalopods (e.g. *Moroteuthis ingens*, Jackson & Mladenov, 1994; Jackson et al., 2004 and *Todarodes pacificus*, Shikata & Shirata, 1999). Since lipid in the digestive gland largely reflects that of the diet (Blanchier & Boucaud-Camou, 1984; Phillips et al., 2001, 2002) the accumulation of large deposits of lipid in the digestive gland may parallel an increased need for protein to fuel the maturation process. For example, since protein and lipid co-occur in prey, the lipid rich digestive gland of *M.ingens* may simply reflect excess lipid resulting from the higher requirements of protein to build up mantle condition preceding spawning. When females cease to feed prior to spawning, they sequester their energy requirements predominantly from protein in the mantle.

3.5.2 Lipid profile of oocyte and egg

The analyses on the lipid dynamics of the ovary relative to maturation, as well as between the ovary and oviduct, underscored the potential importance of the various

lipid classes and fatty acids for embryonic development. While univariate analyses distinguish the relative level or amounts of lipid classes or fatty acids individually, they consider each lipid class or fatty acid independently. Consequently, there is a failure to account for interrelations between the associated lipid classes or fatty acids. The comparative strength of the multivariate analysis (i.e. MANOVA and CVA) is that it permits the relative levels or amounts of lipid classes and fatty acids to be assessed in light of the observed within group correlations. For example, while the univariate ANOVAs demonstrated the overall importance of PL, ST and WE in the mature ovaries (i.e. higher average levels except for ST, and absolute amounts), CVA revealed that the relative levels of all the lipid classes were uniformly increasing, including ST. Canonical variate analysis also showed evidence of a decreasing accumulation in the amount of WE compared to PL and ST, given the observed within maturity stage correlations among the lipid classes. The importance of these classes is further demonstrated by the continued increase of all the lipid classes in the oviduct compared to the ovary, especially ST. This is despite the fact that both the average relative levels and absolute amounts for the classes between the ovary and oviduct remain largely unchanged, except for some variation in the relative levels of winter females.

Although the current literature allows for limited comparisons between lipid class profiles of cephalopod eggs, the predominance of PL in the ovary and oviduct in females of the present study is comparable to the profile of eggs and embryos of the sepiolid *Euprymna tasmanica* (Steer et al. 2004). The overall lipid class profile of eggs in *T. filippovae* mirrored that described for early life stages of the Antarctic squid *Galiteuthis glacialis*, that is, high levels of phospholipid and sterol with limited amounts of reserve lipid (Piatkowski & Hagen, 1994). The well-established role of PL and ST in marine organisms is for the preservation of the structure and function of cellular membranes (Nicol et al., 2004; Tocher, 2003). The role of phospholipid in the ovary is probably associated with the synthesis of the vitellus, as it is a phospholipoprotein (Fujii 1960 cited in Blanchier & Boucaud-Camou, 1984).

Sterol has been shown to be an essential nutrient in higher animals and other invertebrates, that is, as a precursor to hormones, bile salts and vitamin D (Kanazawa, 2001) and as a cell constituent (Cavalli et al., 2001). Cholesterol in the

ommastrephids, *I. coindetii* and *T. eblanae* was related to maturation (Rosa et al., 2005) as it was in *T. filippovae* in the present study. Furthermore, cholesterol ranged from 18-25% of total lipids in the early stages of several species of cephalopods (Navarro & Villanueva, 2000). The fact that the cholesterol as a percentage of total lipids was somewhat lower in the eggs of *T. filippovae* (range 7-9%) than in the young cephalopods studied by Navarro and Villanueva (2000) may suggest a higher requirement for this nutrient after hatching. In addition, canonical variate analysis indicated that ST in *T. filippovae* continued to increase between mature oocytes and ovulation (eggs in the oviduct). It is notable, however, that in the sepiolid *E. tasmanica* there was very little sterol in the eggs or hatchlings (Steer et al., 2004), which may be attributable to a characteristic of this species. Alternatively, it may be that these differences are purely dietary, since some molluscs are characterised by the absence of a *de novo* sterol-synthesising ability. Normally, cephalopods are thought to synthesise cholesterol poorly from acetate and mevalonate (low molecular weight precursors) (Kanazawa, 2001). Sex and species-specific variations in ST levels and amounts (e.g. Rosa et al., 2004, 2005) in some cephalopods is probably due to the exogenous acquisition of their cholesterol requirements, which is influenced by variable life history strategies.

The requirements for reserve lipid in the ovary and oviduct appear limited (range 1-3% WE). Nevertheless, there was a higher relative level and amount of WE in mature oocytes compared to immature. When within group tissue correlations were taken into account, ovulated eggs had increased relative levels of WE compared to oocytes in the mature ovary. Given the importance of PL and ST in the mature egg it is likely that WE may also play a specific role. Wax ester is normally deemed a long-term storage of energy, used when food resources are low (Nicol et al., 2004). While some fish use lipid (especially neutral lipids) in the eggs as nutrient and caloric reserves both during and following embryogenesis (Wiegand et al., 2004), the provision and use of WE in the eggs of *T. filippovae* is unclear. Incubation time is temperature dependent and generally short in the small eggs of some ommastrephids, ranging from 4 to 16 days (Watanabe et al., 1996, Odor & Dawe, 1998). The lipid profile of this species is comparable to small pelagic fish eggs that have high PL levels and short incubation times. Fish eggs with long incubation times normally have substantial neutral lipid reserves, which is predominantly utilized post-hatching

(Tocher, 2003; Tveiten et al., 2004). Although it is unknown if lipid reserves persist in rhynchoteuthion larvae, meager reserves seem characteristic of young cephalopods who funnel their energy into growth (Piatkowski & Hagen, 1994; Navarro & Villanueva, 2000). It is possible that these small reserves are a buffer during the critical transition to exogenous feeding by the rhynchoteuthion hatchlings, which is likely to be some days. However, given that hatchlings may feed on microorganisms including bacteria that are largely protein (Vidal & Haimovici, 1998), and that lipid is digested slowly in cephalopods (O'Dor, 1998), there may also be an alternative role for WE in the eggs.

Neutral lipids in marine animals have been suggested as a significant buoyancy regulating mechanism, by providing an upthrust in water due to a specific gravity lower than that of the surrounding seawater (Falk-Peterson et al, 2000). Adult muscular ommastrephids may have little need for buoyancy due to their ability to remain actively within the water column though energy compensating tactics (see O' Dor & Dawe, 1998; O'Dor et al., 2002). Hatchlings, on the other hand, are likely to be trapped within the epipelagic planktonic habitat as they are essentially functioning similar to a 'blimp' (O'Dor, 2002). After hatching, paralarvae immediately swim upwards, thus concentrating many hatchlings in the more productive surface layer (Bower & Sakurai, 1996). Since rhynchoteuthion larvae are thought to particulate feed (Vidal & Haimovici, 1998), WE may aid in their feeding ecology by promoting their retention within the epipelagic layers.

It was anticipated that higher gonad investment in winter individuals (see chapter 2) might also reflect higher lipid content. Maternal provisioning and selected uptake of lipid may be tied to reproductive tactics in response to the environment as is shown for other marine organisms (e.g. Hagen, 1999). The higher provisioning of lipid in the ovary of autumn females in the present study may be coupled with relatively higher productivity (autumn bloom) at time of capture. Correlation analysis between gonad investment and SSC suggested there was no lag in response time in the three year study examining temporal changes in reproductive parameters (see chapter 2). As demonstrated by Steer et al. (2004) for the sepiolid *E. tasmanica*, lower maternal food rations resulted in correspondingly lower total lipid content in conjunction with lower egg output. In this study the methodology employed precluded an analysis of

egg size and fecundity and therefore it was not possible to determine if lower lipid content in the winter ovaries resulted in a reproductive tradeoff. Nevertheless, this would be of particular interest given that there was no equivalent seasonal difference between the average amounts of lipid content in the oviduct.

In the present study, there appeared to be an overall greater provisioning of PL and less neutral lipids in the autumn oviducts. These discernable differences were evident when assessing variability between the lipid classes of the eggs. Polar lipids are thought to be more stable than neutral lipids and therefore the maternal provision of neutral lipid may be more likely to vary according to maternal diet. These differences may be mainly due to seasonal variation in diet. However, a companion dietary study on *T. filippovae* for the same two seasons resulted in no attributed seasonal differences in any of the lipid classes (Pethybridge, 2004). The exact nature of these seasonal differences in the ovulated egg is unknown. Nevertheless, it may be hypothesized that the greater provisioning of neutral lipid and less PL in the winter females, together with greater gonad investment, may be some form of reproductive tactic whereby the survival of hatchlings are enhanced under certain environmental conditions.

A pattern repeated in other ommastrephids is the small number of fatty acids that dominated the profile of the ovary in *T. filippovae*. In *I. coindettii* and *T. eblanae* gonads, the saturated fraction and the PUFA fraction were predominantly composed of 16:0 and the essential fatty acids DHA and EPA, respectively (Rosa et al., 2005). However, MUFA differed in the dominant fatty acids. *Todarodes filippovae* was dominated by 20:1(n-9)c whereas the *I. coindettii* and *T. eblanae* ovaries were dominated by 18:1(n-9). The digestive glands of all species were also high in these fatty acids. It may be reasonable to assume that these differences are dietary due to the fact that lipid from prey is largely unmodified in squid digestive glands (Phillips et al., 2001; 2002).

3.5.3 Role of essential fatty acids

The PUFA fraction of fish eggs is characterised by the preponderance of n-3 unsaturated fatty acids, most notably DHA and EPA (Tveiten et al., 2004). Since

many marine species are unable to synthesise these EFA *de novo*, they must acquire them from their diet (e.g. Tocher, 2003; Phleger et al., 2002). The marine environment is rich in EPA and DHA, originating at the base of the food chain in the form of diatoms and flagellates respectively. These EFA are then transferred intact up the food chain to higher predators (Tocher, 2003). The importance of these essential fatty acids has been highlighted in the manipulation of broodstock diets. Furthermore, the EFA, EPA and arachidonic acid (ARA) are important in the structure and function of cell membranes (Cavelli et al., 2001; Tveiten et al., 2004) as well as precursors of prostaglandins (Bell & Sargent, 2003; Lilly and Bottino 1981 cited in Cavalli et al., 2001). The essentiality of ARA has largely been overlooked possibly due to the low requirements of this EFA in marine organisms (Bell et al., 2003). However, ARA is cited as important for promoting growth and survival and regulation of the immune system in larval fish (Bell & Sargent, 2003).

Recent research has emphasised a vital role for DHA in the functioning of neural tissue (brain and eye) in marine fish larvae and juveniles (Ishizaki et al., 2001; Tocher, 2003). Cephalopods are well known for their complex vertebrate like nervous system, the most sophisticated of all invertebrates (Budelmann, 1994). It is therefore likely that provision of these fatty acids will also be critical for the survival of cephalopods. An essential survival mechanism in cephalopods is simultaneous innervation of the mantle, fins and retractor muscles important in escape responses to predators. This is possible through the conduction of nerve impulses in the giant axon, which is very much larger than mammalian fibers (Budelman, 1994; Mangold et al., 1998). The complicated behaviour and defence system characteristic of cephalopods is facilitated by rapid and neurally-controlled body patterning, processed via the eyes and optic lobes of the brain (Hanlon & Messenger, 1996). Moreover, the voracious appetite and predatory behaviour of cephalopods is facilitated by a large vertebrate-like visual system (Budelman, 1994).

The importance of essential fatty acids in the eggs of *T. filippovae,* that is likely to be implicated in the embryonic development and growth of juvenile *T. filippovae*, confirms the statement by Boyle et al (2001) that there is expected to be a high provision of essential fatty acids in cephalopod eggs. The considerable proportion of DHA and EPA as a percentage of total fatty acids (~25 and 10% respectively) in the

yolk of *Illex illecebrosus* eggs (Boyle et al., 2001) as well as in the oviducts of *T. fillippovae* in this study (~21% and 9% respectively) signifies the relative importance of these fatty acids. Although higher DHA/EPA and EPA/AA ratios were evident in mature individuals, the importance of DHA and EPA was not directly apparent when comparing observed maturational differences in the relative levels. Average levels of DHA did not vary, and levels of EPA were lower in mature ovaries than immature ovaries. The percentage accumulation of DHA decreased when the observed within maturity stage correlations were considered. However, the univariate ANOVAs demonstrated that all 8 fatty acids, including DHA and EPA, showed a tendency to increase in content with maturity. Canonical variate analysis showed that in mature individuals the accumulation of DHA had decreased in comparison to the accumulation of EPA, given the observed within maturity stage correlations among these fatty acids.

The importance of n-3 PUFA levels, that is, EFA in some fish eggs and embryos has been related to egg viability. Broodstock diets with enhanced levels of essential nutrients have resulted in improved egg morphology and hatching rates (Izquierdo et al., 2001). In the present study, egg quality regardless of seasonal differences in total lipid content, gonad investment and individual body size was largely conserved. The only seasonal variation observed was a decrease in the accumulation of DHA and EPA in the autumn females compared to that observed for winter. While lipid content in *E. tasmanica* was significantly less in females apportioned low food rations, there was no concomitant decrease in egg quality as it relates to lipid class or fatty acids. Steer et al (2004) established that females appear to trade fecundity and egg size in preference to egg quality. Gowland et al. (2002) suggest that the rate of embryonic abnormality may reflect quality of yolk provision. They suggest that environmental conditions may force the responses observed in egg quality. It is likely that seasonal differences in total lipid content in *T. filippovae*, most likely in response to differing food conditions at time of capture, may also drive some kind of reproductive tradeoff. Koueta et al. (2002) documented the significance of n-3 PUFA in the improved growth of juvenile cuttlefish, which was sustained throughout the experiment. Similarly, Navarro and Villanueva (2000) recognized the importance of PUFA for the growth requirements of several juvenile species of cephalopods. The overall seasonal preservation of the essential fatty acids observed in the eggs from

this study, along with that documented for *E. tasmanica* when subjected to varying dietary rations (Steer et al., 2004) is indicative of a reproductive advantage. A PUFA enriched diet may enhance central nervous system development and therefore increase feeding efficiencies through enhanced predatory functioning (Koueta et al., 2002). High feeding efficiencies are essential for squid hatchlings that are sophisticated visual feeders (Boyle et al., 2001) and may be responsible in determining survival following the critical transition to exogenous feeding (Vidal et al., 2002). Therefore, it is most likely that females would employ a reproductive tradeoff to ensure that potential mortality is decreased under less than optimal conditions.

3.6 Conclusions

This study provided an assessment of the role of lipid in the maturation of a poorly understood ommastrephid species *T. filippovae*. The role of lipid in relation to maturation for *T. filippovae* is not likely to be as an energy reserve but one of maternal provisioning to supply essential nutrition to the egg and subsequent embryo. This is in marked contrast to many other marine organisms whereby lipid stores are a buffer against pulsed seasonal food resources, thus enabling reproduction to be fuelled independently of environmental conditions (see Hagen, 1999). The 'live-for-now' lifestyle of squid precludes the requirement of large lipid reserves. To do so would sacrifice mobility. A predominantly amino acid based metabolism allows juveniles to absorb protein and funnel all their energy into growth, thus in effect wasting much of the lipid (O'Dor, 1998). Given the extreme reproductive plasticity observed in squid, there is likely to be varying levels of tradeoffs in response to an unpredictable environment. Further research is required to elucidate the reproductive plasticity of squid in relation to maternal provisioning of lipid.

CHAPTER FOUR
GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

The application of both traditional techniques, such as morphometric analysis and the relatively newer technique of lipid and fatty acid analysis has revealed that the reproduction of *Todarodes filippovae* is closely linked to the environment. This has been previously suggested for other ommastrephid species (O'Dor & Dawe, 1998; McGrath-Steer, 2004), and does highlight the marked plasticity of this species. Population dynamics may predominantly be influenced by phenotypic plasticity (Boyle & Boletsky, 1996), and it is likely that the variability in condition and reproductive parameters documented over this three year study was also a phenotypic response to a variable environment. However, oceanographic processes are complex, and it is often difficult to extract specific causes in relation to biological responses. The variability in condition and gonad investment observed in this study was probably not due to any one factor but to the interaction between biotic and abiotic factors.

Positive correlations between both gonad investment and somatic condition with sea surface colour (SSC) suggest that differences in these parameters may be causally related and therefore account for some of the temporal variability. It was not clear however, if the large peak in SSC in the second year of the study was driving the relationships. Interesting questions exist in relation to the exact nature of the role of food in determining reproductive success. Many researchers have implicated food as an important determinant in the growth process, and by extension maturation, since they are inextricably linked. However, limited research has examined the important impact of biotic factors in relation to maternal provisioning for reproduction. Since the energy available for reproductive investment is essential for the survival and proper development of hatchlings, these effects can potentially affect recruitment processes (Heyer et al., 2001).

This research would not have been possible without the co-operation and support of the deep-water trawl fishery, although a major constraint in this study was the sampling limitations imposed by the fisheries. Due to quotas being met in the first half of the year, it was not possible to obtain winter or spring samples from southern

Tasmania. For the most part, winter samples were collected from eastern Tasmania and therefore seasonal winter samples may be confounded by location. Similarly, a missing winter cell in the second year of the study precluded a complete seasonal analysis for that year. This limited sampling regime may account for the surprising lack of a temperature signal, since only summer and autumn samples were used in the correlation analysis. Alternatively, since temperature is highly influential in the early stages of squid development (Forsythe, 1993), the effect may be less pronounced at time of capture. Further analyses with more intense annual and seasonal sampling at various locations in this region in association with environmental (such as SST and SSC) and biological (such as prey availability and abundance) variables peculiar to this region, may help to determine the mechanisms driving the variation in condition and reproductive strategies found in this study.

The spawning population off Eastern Tasmania (which were smaller and had greater gonad investment) compared to southern Tasmania may be a different population or a result of phenotypic plasticity. Very little is known about how the reproductive cycle of this species is linked to the oceanography of this region, i.e., if the close link between other ommastrephids and current systems is also applicable for *T. filippovae*. Therefore, it is only possible to hypothesize as to where these females originated. Undoubtedly, a genetic study involving large-scale sampling in and around all Tasmanian waters may shed some light on this question.

This research further highlighted the adaptive lifestyle of *T. filippovae* that enables females to exploit opportunities when and wherever they occur. Multiple spawning in this species may be a key survival strategy not only for the female but for the offspring as well. The survival of some young is likely ensured under varying conditions due to the females being able to spread the risk associated with a patchy pelagic environment. The flexibility in spawning tactics in response to productivity was probably most evident in the shift towards the terminal end of the multiple versus terminal spawning continuum, for the autumn individuals in the second year of the study. Although this shift was not large, it does suggest that a species may vary its reproductive tactics within an overall strategy depending on the current environment.

The high responsiveness to the environment and the opportunistic lifestyle of this species is probably best reflected in its limited need for lipid as an energy source to fuel maturation. This highly mobile species is able to actively feed and conserve condition with no obvious depletion of energy reserves under various environmental constraints. Most likely, the excess lipid in the digestive gland was a response to a greater need for protein to fuel the maturation process. The exact role of this 'excess' lipid in the digestive gland is currently unknown for this species. Lipid analysis on the content of the intestine may help to reveal if the lipid is being excreted, as Semmens (1998) found for two loliginid species. Digestive gland lipid may assist with buoyancy as *T. filippovae* lives at great depth. Although given the locomotory tradeoff that accompanies buoyancy, this role may not be significant (O'Dor et al., 2002) for an active muscular ommastrephid species. Although there was no evidence of a reproductive tradeoff at the whole animal level, a study to determine if there was any change in the muscle fibres at the cellular level may reveal some modification. However, this would most likely be minor as found by McGrath-Steer (2004) for the ommastrephid *Nototodarus gouldi.*

Lipid is likely to play an important role in embryonic development, and by inference, in the growth and development of the rhynchoteuthion. The importance of lipid, in particular PUFA, has recently been implicated for young cephalopods (Navarro & Villanueva, 2000) and in the eggs of other ommastrephids (Rosa et al., 2005) and is confirmed in this study. Given the limited requirements and slow digestibility of lipids by cephalopods generally, the role of wax ester (a long-term storage lipid) in the eggs of this species deserves further attention. A comparison between the eggs and various stages of rhynchotethion hatchlings would highlight if lipid were being used as an energy reserve.

The overall quality of the egg in terms of fatty acids in this species was conserved between seasons. However, changes in the lipid content and lipid classes of the maturing oocytes or eggs (in the oviduct) may be reflective of varying conditions at time of capture in terms of maternal food resources. These fluctuations may also represent maternal responses to the environment in terms of maternal provisioning to the egg, ensuring greater growth and survival in a patchy pelagic habitat. To define the role that the environment is forcing on the reproductive strategy of this species

requires a combination of analyses including lipid content and composition of the egg, fecundity (egg versus size), morphometrics and lipid signature profiles to ascertain prey profiles over a number of seasons and years in conjunction with environmental variables such as SST and SSC.

This study on the reproductive plasticity of female individuals of *T. filippovae* underscores that this species is highly adapted to its environment. They grow, reproduce and compete successfully in their pelagic environment despite their short life spans and lack of energetic reserves. Their predatory and opportunistic 'live for now' lifestyle ensures their survival. *T. filippovae* is in fact yet another good cephalopod example of the ultimate law of biology 'whatever works works' (O'Dor, 1998).

LITERATURE CITED

Abitia-Cardenas LA, Muhlia-Melo A, Cruz-Escalona V, Galvan-Magana F (2002) Trophic dynamics and seasonal energetics of striped marlin *Tetrapturus audax* in the southern Gulf of California, Mexico. Fish Res 57:287-295

Agnew DJ, Beddington JR, Hill SL (2002) The potential use of environmental information to manage squid stocks. Can J Fish Aquat Sci 59:1851-1857

Anderson CIH, Rodhouse PG (2001) Life cycles, oceanography and variability: ommastrephid squid in variable oceanographic environments. Fish Res 54:133-143

Arkhipkin AI (2004) Diversity in growth and longevity in short-lived animals: squid of the suborder Oegopsina. Mar Freshw Res 55:341-355

Arkhipkin AI, Bjørke H (1999) Ontogenetic changes in morphometric and reproductive indices of the squid *Gonatus fabricii* (Oegopsida, Gonatidae) in the Norwegian Sea. Polar Biol 22:357-365

Begon M, Harper JL, Townsend CR (1986) Ecology. Blackwell Sci Publ, Oxford, 876pp

Bell G (1980) The costs of reproduction and their consequences. Am Nat 116:45-76

Bell JG, McEvoy LA, Estevez A, Shields RJ, Sargent JR (2003) Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture 227:211-220

Bell JG, Sargent JR (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218:491-499

Blanchier B, Boucaud-Camou (1984) Lipids in the digestive gland and the gonad of immature and mature *Sepia officinalis* (Mollusca: Cephalopoda). Mar Biol 80:39-43

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917

Boletzky, Sv (2003) Biology of early life stages in cephalopod mollusks. Adv Mar Biol 44:143-203

Boucaud-Camou E, Yim M, Tresgots A (1985) Feeding and digestion of young *Sepia officinalis* L. (Mollusca: Cephalopoda) during post hatching development. Vie Milieu 35:263-266

Bower JR, Sakurai Y (1996) Laboratory observations on *Todarodes pacificus* (Cephalopoda: Ommastrephidae) egg masses. Am Malac Bull 13:65-71

Boyle PR, Boletzky Sv (1996) Cephalopod populations: definition and dynamics. Phil Trans R Soc Lond B 351:985-1002

Boyle PR, Noble L, Emery AM, Craig S, Black KD, Overnell J (2001) Development and hatching in cephalopod eggs: a model system for partitioning environmental and genetic effects on development. In: Atkinson D, Thorndyke M (eds) Environment and animal development : genes, life histories and plasiticity. BIOS Scientific Publishers Ltd, Oxford, pp251-267

Boyle PR, Pierce GJ, Hastie LC (1995) Flexible reproductive strategies in the squid *Loligo forbesi*. Mar Biol 121:501-508

Brown, ML, Murphy BR (2004) Seasonal dynamics of direct and indirect condition indices in relation to energy allocation in largemouth bass *Micropterus salmoides* (Lacèpede). Ecol Freshwat Fish 13:23-36

Budelmann BU (1994) Cephalopod sense organs, nerves and the brain: adaptations for high performance and life style. Mar Fresh Behav Physiol 25:13-33

Burton HR, van den Hoff J (2002) Humans and the southern elephant seal (*Mirounga leonina*). Aust Mamm 24:127-140

Butler J, Fuller D, Yaremko M (1999) Age and growth of market squid (*Loligo opalescens*) off California during 1998. CalCOFI Rep, 40:191-195

Caddy JF, Rodhouse PG (1998) Cephalopod and groundfish landings: evidence for ecological change in global fisheries? Rev Fish Biol Fisheries

Castro BG, Garrido JL, Sotelo CG (1992) Changes in composition of digestive gland and mantle muscle of the cuttlefish *Sepia officinalis* during starvation. Mar Biol 114:11-20

Cavalli RO, Tamtin M, Lavens P, Sorgeloos P (2001) Variations in lipid classes and fatty acid content in tissues of wild *Macrobrachium rosenbergii* (de Man) females during maturation. Aquaculture 193:311-324

Cherel Y, Klages N (1997) A review of the food of albatrosses. In: Robertson G, Gales R (eds) Albatross biology and conservation. Surrey Beatty & Sons, Chipping Norton, pp 113-136

Clarke MR (1996) Cephalopods as prey. III. Cetaceans. Phil Trans R Soc Lond B 351:1053-1065

Collins MA, Burnell GM, Rodhouse PG (1995) Reproductive strategies of male and female *Loligo forbesi* (Cephalopoda: Loliginidae). J mar biol Ass UK 75:621-634

Craig SR, MacKenzie DS, Jones G, Gatlin DM III (2000) Seasonal changes in the reproductive condition and body composition of free-ranging red drum, *Sceaenops ocellatus*. Aquaculture 190:89-102

Croxall JP, Prince (1996) Cephalopods as prey. I. Seabirds. Phil Trans R Soc Lond B 351:1023-1043

Cubillos LA, Arcos DF, Bucarey DA, Canales MT (2001) Seasonal growth of small pelagic fish off Talcahuano, Chile (37°S, 73°W): a consequence of their reproductive strategy to seasonal upwelling. Aquat Living Res 14:115-124

Dawe EG, Colbourne EB, Drinkwater KF (2000) Environmental effects on recruitment of short-finned squid (*Illex illecebrosus*). ICES J Mar Sci 57:1002-1013

Duarte CM, Alcaraz M (1989) To produce many small or few large eggs: a size-independent reproductive tactic of fish. Oecologia 80:401-404

Dunning (1998a) *Todarodes pacificus pusillus* new subspecies (Cephalopoda: Ommastrephidae) from northern Australia. Mem Mus Vict 49(1):149-157

Dunning (1988b) Distribution and comparative life history studies of deepwater squid of the family *Ommastrephidae* in Australasian waters. PhD Thesis, University of Queensland, 288pp.

Dunning MC (1998) Zoogeography of arrow squids (Cephalopoda: Ommastrephidae) in the Coral and Tasman Seas, Southwest Pacific. Smith Cont Zool 586: 435-453

Dunning MC (1993) Summer populations of *Ommastrephes bartramii* (Lesueur,1821) and *Todarodes filippovae* Adam, 1975 (Cephalopoda: Ommastrephidae) from the Tasman Sea In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 97-118

Dunning MC, Brandt SB (1985) Distribution and life history of deepwater squid of commercial interest from Australia. Aust J Mar Freshwat Res 36:343-359

Dunning MC, Wormuth JH (1998) The ommastrephid squid genus *Todarodes*: a review of systematics, distribution, and biology (Cephalopoda: Teuthoidea). Smith Cont Zool 586: 385-391

Elliott JK, Leggett WC (1998) Larval condition and vulnerability to predation: an analysis based on mixed-prey experiments. Can J Fish Aquat Sci 55:626-630

Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57:178-191

Fenton GE, Short SA, Ritz DA (1991) Age determination of orange roughy, *Hoplostethus atlanticus* (Pisces: Tachichthyidae) using ^{210}Pb:^{226} disequilibria. Mar Biol 109:197-202

Fields WG (1965) The structure, development, food relations, reproduction, and life history of the squid *Loligo opalescens* Berry. Calif Dep Fish Game Fish Bull 131:1-108

Forsman A (2001) Clutch size versus clutch interval: life history strategies in the colour-polymorphic pygmy grasshopper *Tetrix subulata*. Oecologia 129:357-366

Forsythe JW (1993) A working hypothesis of how seasonal temperature change may impact the field growth of young cephalopods. In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 133-143

Forsythe JW (2004) Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice. Mar Freshwat Res 55: 331-339

Forsythe JW, Walsh LS, Turk PE (2001) Impact of temperature on juvenile growth and age at first egg-laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. Mar Biol 138:103-112

Gowland FC, Moltschaniwskyj NA, Steer MA (2002) Description and quantification of developmental abnormalities in natural *Sepioteuthis australis* spawning population (Mollusca: Cephalopoda). Mar Ecol Prog Ser 243:133-141

Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82:1473-1483

Guerra A, Castro BG (1994) Reproductive-somatic relationships in *Loligo gahi* (Cephalopoda: Loliginidae) from the Falkland Islands. Antarct Sci 6:175-178

Hagen W (1999) Reproductive strategies and energetic adaptations of polar zooplankton. Inv Reprod Dev 36:25-34

Hanlon RT, Messenger JB (1996) Cephalopod behaviour. Cambridge University Press, Cambridge, 232pp.

Harman RF, Young RE, Reid SB, Mangold KM, Suzuki T, Hixon RF (1989) Evidence for multiple spawning in the tropical oceanic squid *Stenoteuthis oualaniensis* (Teuthoidea: Ommastrephidae). Mar Biol 101:513-519

Harris GP, Griffiths FB, Clementson LA, Lyne V, Van der Doe H (1991) Seasonal and interannual variability in physical processes, nutrient cycling and the structure of the food chain in Tasmanian shelf waters. J Plankton Res 13:109-131

Harris G, Nilsson C, Clementson L, Thomas D (1987) The water masses of the east coast of Tasmania: seasonal and interannual variability and the influence on phytoplankton biomass and productivity. Aust J Mar Freshw Res 38:569-90

Hatanaka H, Kawahara S, Uozumi Y (1985) Comparison of life cycles of five ommastrephid squids fished by Japan: *Todarodes pacificus*, *Illex illecebrosus*, *Illex argentinus*, *Notodarus sloani sloani* and *Nototodarus sloani gouldi*. NAFO Sci Coun Studies 9:59-68

Hatfield EMC (2000) Do some like it hot? Temperature as a possible determinant of variability in the growth of the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae). Fish Res 47:27-40

Hatfield EMC, Cadrin SX (2002) Geographic and temporal patterns in size and maturity of the longfin inshore squid (*Loligo pealeii*) off the northeastern United States. Fish Bull 100:200-213

Hatfield EMC, Rodhouse PG (1992) Production of soma and gonad in maturing female *Illex argentinus* (Mollusca:Cephalopoda). J mar biol Ass UK 72:281-291

Hernández MD, Egea MA, Rueda FM, Martinez FJ, Garcia Garci'a B (2003) Seasonal condition and body composition changes in sharpsnout seabream (*Diplodus puntazzo*) raised in captivity. Aquaculture 220:569-580

Heyer CJ, Miller TJ, Binkowski FP, Caldarone EM, Rice JA (2001) Maternal effects as a recruitment mechanism in Lake Michigan yellow perch (*Perca flavescens*). Can J Fish Aquat Sci 58:1477-1487

Ho JD, Moltschaniwskyj NA, Carter CG (2004) The effect of variability in growth on somatic condition and reproductive status in the southern calamary. Mar Freshw Res 55:423-428

Ichii T, Mahapatra K, Sakai M, Inagake D, Okada Y (2004) Differing body size between the autumn and the winter-spring cohorts on neon flying squid (*Ommastrephes bartramii*) related to the oceanographic regime in the North Pacific: a hypothesis. Fish Oceanogr 13:295-309

Ikeda I, Sakurai Y, Shimazaki K (1993) Maturation process of the Japanese common squid *Todarodes pacificus* in captivity. In: Okutani T, O'Dor R, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 179-187

Ishizaki Y, Masuda R, Uematsu K, Shimizu K, Arimoto M, Takeuchi T (2001) The effect of dietary docosahexaenoic acid on schooling behaviour and brain development in larval yellowtail. J Fish Biol 58:1691-1703

Izquierdo MS, Fernández-Palacios H, Tacon AGJ (2001) Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197:25-42

Jackson GD (1993) Seasonal variation in reproductive investment in the tropical loliginid squid *Loligo chinensis* and the small tropical sepioid *Idiosepius pygmaeus*. Fish Bull 91:260-270

Jackson GD (2004) Advances in defining the life histories of myopsid squid. Mar Freshw Res 55:357-365

Jackson GD, Domeier ML (2003) The effects of an extraordinary El Niño/La Niña event on the size and growth of the squid *Loligo opalescens* off Southern California. Mar Biol 142:925-935

Jackson GD, Forsythe JW, Hixon RF, Hanlon RT (1997) Age, growth and maturation of *Lolliguncula brevis* (Cephalopoda: loliginidae) in the Northwestern Gulf of Mexico with a comparison of length-frequency vs. statolith age analysis. Can J Fish Aquat Sci 54:2907-2919.

Jackson GD, McGrath Steer B, Wotherspoon S, Hobday AJ (2003) Variation in age, growth and maturity in the Australian arrow squid *Nototodarus gouldi* over time and space – what is the pattern? Mar. Ecol. Prog. Ser. 264: 57-71

Jackson GD, McKinnon JF, Lalas C, Ardern R, Buxton N (1998) Food spectrum of the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) in New Zealand waters. Polar Biol 20:56-65

Jackson GD, Mladenov PV (1994) Terminal spawning in the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae). J Zool Lond 234:189-201

Jackson GD, Moltschaniwskyj (2002) Spatial and temporal variation in growth rates and maturity in the Indo-Pacific squid *Sepioteuthis lessoniana* (Cephalopoda: loliginidae). MarBiol 140:747-754

Jackson GC, O'Dor (2001) Time, space and the ecophysiology of squid growth, life in the fast lane. Vie Milieu 51:205-215

Jackson GD, Semmens JM, Phillips KL, Jackson CH (2004) Reproduction in the deepwater squid *Moroteuthis ingens*, what does it cost? Mar Biol 145:905-916

Kanazawa A (2001) Sterols in marine invertebrates. Fish Sc 67:997-1007

Kirkendall LR, Stenseth NC (1985) On defining "breeding once". Am Nat 125:189-204

Klages NTW (1996) Cephalopods as prey. II. Seals. Phil Trans R Soc Lond B 351:1045-1052

Koueta N, Boucaud-Camou E, Noel B (2002) Effect of enriched natural diet on survival and growth of juvenile cuttlefish *Sepia officinalis* L. Aquaculture 203:293-310

Laine P, Rajasilta M (1999) The hatching success of Baltic herring eggs and its relation to female condition. Jnl Exp Mar Biol Ecol 237:61-73

Lambert Y, Dutil J-D (2000) Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. Can J Fish Aquat Sci 57:815-825

Laptikhovsky VV, Nigmatullin CM (1993) Egg size, fecundity, and spawning in females of the genus *Illex* (Cephalopoda: Ommastrephidae). ICES J mar Sci 50:393-403

Lee PG, (1994) Nutrition of cephalopods: fueling the system. Mar Fresh Behav Physiol 25: 35-51

Lewis AR, Choat JH (1993) Spawning mode and reproductive output of the tropical cephalopod *Idiosepius pygmaeus*. Can J Fish Aquat Sci 50:20-28

Lipinski MR (1979) Universal maturity scale of the commercially important squids. The results of maturity classification of the *Illex illecebrosus* populations for the years 1973-77. ICNAF Res Doc 79/2/38, Serial 5364, International Commission for the Northwest Atlantic Fisheries, Dartmouth, Canada

Lloret J, de Sola LG, Souplet A, Galzin, R (2002) Effects of large-scale habitat variability on condition of demersal exploited fish in the north-western Mediterranean. ICES J Mar Sci 59:1215-1227

Mangold K, Clarke MR, Roper CFE (1998) Class Cephalopoda. In: Beesley PL, Ross GJB, Wells A (eds) Mollusca: The Southern Synthesis. Fauna of Australia. Vol. 5. CSIRO Publishing, Melbourne, pp451-484

Mangold KM, Young RE, Nixon M (1993) Growth versus maturation in cephalopods. In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 697-703

Markaida U, Sosa-Nishizaki O (2001) Reproductive biology of jumbo squid *Dosidicus gigas* in the Gulf of California, 1995-1997. Fish Res 54:63-82

Marshall CT, Frank KT (1999) The effect of interannual variation in growth and condition on haddock recruitment. Can J Fish Aquat Sci 56:347-355

Maxwell MR, Hanlon RT (2000) Female reproductive output in the squid *Loligo pealeii*: multiple egg clutches and implications for a spawning strategy. Mar Ecol Prog Ser 199:159-170

Melo YC, Sauer WHH (1999) Confirmation of serial spawning in the chokka squid *Loligo vulgaris reynaudii* off the coast of South Africa. Mar Biol 135:307-313

McGrath-Steer (2004) Flexibility in the reproductive strategies of *Nototodarus gouldi*, Macoy 1888 (Cephalopoda. Ommastrepidae) from southeastern Australia. PhD thesis, University of Tasmania, 109pp

McGrath BL, Jackson GD (2002) Egg production in the arrow squid *Nototodarus gouldi* (Cephalopoda: Ommastrephidae), fast and furious or slow and steady? Mar Biol 141:699-706

McGrath Steer BL, Jackson GD (2004) Temporal shifts in the allocation of energy in the arrow squid, *Nototodarus gouldi*: sex-specific responses. Mar Biol 144:1141-1149

Moltschaniwskyj NA (1995) Multiple spawning in the tropical squid *Photololigo* sp.: what is the cost in somatic growth? Mar Biol 124:127-135

Moltschaniwskyj NA, Semmens (2000) Limited use of stored energy reserves for reproduction by the tropical loliginid squid *Photololigo* sp. J Zool Lond 251:307-313

Mourente G, Megina C, Díaz-Salvago (2002) Lipids in female northern bluefin tuna (*Thunnus thynnus thynnus* L.). Fish Physiol Biochem 24:351-363

Myers RA, Worm B (2003) Rapid worldwide depletion of predatory fish communities. Nature 423:280-283

National Oceans Office (2002) Ecosystems - nature's diversity, the South-East Regional Marine Plan. National Oceans Office, Hobart, 214pp

Navarro JC, Villanueva R (2000) Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. Aquaculture 183:161-177

Nesis KN (1987) Cephalopods of the world. TFH Publications, Neptune City, 351pp

Nicol S, Virtue P, King R, Davenport SR, McGaffin AF, Nichols P (2004) Condition of *Euphausia crystallorophias* off East Antarctica in winter in comparison to other seasons. Deep-Sea Res II 51:2215-2224

O'Dor RK (1992) Big squid in big currents. S Afr J mar Sci 12:225-235.

O'Dor RK (1998) Squid life history strategies. FAO Fish Tech Pap 376:233-268

O'Dor RK (2002) Telemetered cephalopod energetics: swimming, soaring, and blimping. Int Comp Biol 42:1065-1070

O'Dor RK, Adamo S, Aitken JP, Andrade Y, Finn J, Hanlon RT, Jackson GD (2002) Currents as environmental constraints on the behavior, energetics and distribution of squid and cuttlefish. Bull Mar Sci 71:601-617

O'Dor RK, Balch N (1985) Properties of *Illex illecebrosus* egg masses potentially influencing larval oceanographic distribution. NAFO Sci Coun Studies 9:69-76

O'Dor RK, Dawe EG (1998) *Illex illecebrosus*. FAO Fish Tech Pap 376:77-104

O'Dor RK, Helm P, Balch N (1985) Can rhynchoteuthions suspension feed? (Mollusca: Cephalopoda). Vie Milieu 35:267-271

O'Dor RK, Mangold K, Boucher-Rodoni R, Wells MJ, Wells J (1984) Nutrient absorption, storage and remobilization in *Octopus vulgaris*. Mar Behav Physiol 11:239-258

O'Dor RK Rodhouse PG, Dawe EG (1997) Squid recruitment in the genus *Illex*. In: Hancock, DA, Smith DC, Grant A, Beumer JP (eds) Developing and sustaining world fisheries resources. CSIRO Publishing, Collingwood, pp 116-121

O'Dor RK, Webber DM (1986) The constraints on cephalopods: why squid aren't fish. Can J Zool 64:1591-1605

O'Dor RK, Wells MJ (1987) Energy and nutrient flow. In: Boyle PR (ed) Cephalopod life cycles Vol II, Academic Press, London pp109-133

Ouellet P, Lambert Y, Be'rube' I (2001) Cod egg characteristics and viability in relation to low temperature and maternal nutritional condition. ICES J Mar Sci 58:672-686

Parrish CC, Thompson RJ, Deibel D (2005) Lipid classes and fatty acids in plankton and settling matter during the spring bloom in a cold ocean coastal environment. Mar Ecol Prog Ser 286:57-68

Pauly D, Christensen V, Dalsgaard J, Froese R, Torres F Jr (1998) Fishing down marine food webs. Science 279:860-863

Pawitan Y (2001) In all likelihood: statistical modelling and inference using likelihood. Oxford University Press Inc., New York, 528pp

Pecl G (2001) Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids. Mar Biol 138:93-101

Pecl, GT (2004) The *in situ* relationships between season of hatching, growth and condition in the southern calamary, *Sepioteuthis australis*. Mar Freshw Res 55:429-438

Pecl, GT, Moltschaniwskyj NA, Tracey, SR, Jordan AR (2004) Inter-annual plasticity of squid life history and population structure: ecological and management implications. Oecologia 139:515-524

Phillips KL (2003) A dietary study of *Moroteuthis ingens* and other Southern Ocean squid species: combined stomach contents and fatty acid analyses. PhD Thesis, University of Tasmania, 161pp

Phillips KL, Jackson GD, Nichols PD (2001) Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Islands: stomach contents and fatty acid analyses. Mar Ecol Prog Ser 215:179-189

Phillips KL, Nichols PD, Jackson GD (2002) Lipid and fatty acid composition of the mantle and digestive gland of four Southern Ocean squid species: implications for food-web studies. Antarct Sci 14:212-220

Phleger CF, Nelson MN, Groce AK, Cary CS, Coyne KJ, Nichols PD (2005) Lipid composition of deep-sea hydrothermal vent tubeworm *Riftia pachyptila*, crabs *Munidopsis subsquamosa* and *Bythograea thermydron*, mussels *Bathymodiolus* sp. and limpets *Lepetodrilus* spp. Comp Biochem Physiol B 141:196-210

Piatkowski U, Hagen W (1994) Distribution and lipid composition of early life stages of the cranchiid squid *Galiteuthis glacialis* (Chun) in the Weddell Sea, Antarctica. Ant Sci 6:235-239

Pethybridge H (2004) Diet of an ommastrephid squid, *Todarodes filippovae* from east and south Tasmania: stomach content and signature lipid analysis. Honours thesis, University of Tasmania, 79pp

Poizat G, Rosecchi E, Crivelli AJ (1999). Empirical evidence of a trade-off between reproductive effort and expectation of future reproduction in female three-spined sticklebacks. Proc Roy Soc London B 266:1543-1548

Pörtner H-O, Zielinski S (1998) Environmental constraints and the physiology of performance in squids. S Afr J mar Sci 20:207-221

Prince JD (2001) Ecosystem of the South East Fishery (Australia), and fisher lore. Mar Freshwat Res 52:431-449

Raymond J-F, Himmelman JH, Guderley HE (2004) Sex differences in biochemical composition, energy content and allocation to reproductive effort in the brooding sea star *Leptasterias polaris*. Mar Ecol Prog Ser 283:179-190

Rideout RM, Trippel, Litvak MK (2005) Effects of egg size, food supply and spawning time on early life history success of haddock *Melanogrammus aeglefinus*. Mar Ecol Prog Ser 285:169-180

Robin JP, Denis V (1999) Squid stock fluctuations and water temperature: temporal analysis of English Channel Loliginidae. J Appl Ecol 36:101-110

Rocha F, Guerra A, González AF (2001) A review of reproductive strategies in cephalopods. Biol Rev 76:291-304

Rodhouse PG (1998) *Todarodes filippovae* in the Southern Ocean: an appraisal for exploitation and management. Okutani T (ed) Large pelagic squids. Japan Marine Fishery Resources Research Center, Tokyo, pp207-215

Rodhouse PG (2001) Managing and forecasting squid fisheries in variable environments. Fish Res 54:3-8

Rodhouse PG, Elvidge CD, Trathan PN (2001) Remote sensing of the global light-fishing fleet: an analysis of interactions with oceanography, other fisheries and predators. Adv Mar Biol 39:261-303

Rodhouse PG, Nigmatullin CH M (1996) Role as consumers. Phil Trans R Soc Lond B 351:1003-1022

Roeleveld MA (1989) The occurrence of two species of squid *Todarodes* in the Benguela System. S Afr J Sci 85: 659-663

Roper CFE, Sweeney MJ, Nauen CE (1984). Cephalopods of the world. FAO Fish Synop 125 Vol 3, 277pp

Rosa R, Costa PR, Bandarra N, Nunes ML (2005) Changes in tissue biochemical composition and energy reserves associated with sexual maturation in the ommastrephid squids *Illex coindetii* and *Todaropsis eblanae*. Biol Bull 208:100-113

Rosa R, Costa PR, Nunes ML (2004) Effect of sexual maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca: Cephalopoda). Mar Biol 145:563-574

Sakurai Y, Kiyofuji H, Saitoh S, Goto T, Hiyama Y (2000) Changes in inferred spawning areas of *Todarodes pacificus* (Cephalopoda: Ommastrephidae) due to changing environmental conditions. ICES J mar Sci 57:24-30

Sargent J, Bell G, McEvoy L, Tocher D, Estevez A (1999) Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177:191-199

Sauer, WHH, Melo YC, Boletzky S (2002) Squid and anchovy: similar subjects, different schools. Bull Mar Sci 71:771-782

Seibel BA, Goffredi SK, Thuesen EV, Childress JJ, Robison BH (2004) Ammonium content and buoyancy in midwater cephalopods. J Exp Mar Biol Ecol 313:375-387

Seibel BA, Hochberg, FG, Carlini DB (2000) Life history of *Gonatus onyx* (Cephalopoda: Teuthoidea): deep-sea spawning and post-spawning egg care. Mar Biol 137:519-526

Semmens JM (1998) An examination of the role of the digestive gland of two loliginid squids, with respect to lipid: storage or excretion? Proc R Soc Lond B 265:1685-1690

Semmens JM, Moltschaniwskyj NA, Alexander CG (1995) Effect of feeding on the structure of the digestive gland of the tropical sepioid *Idiosepius pygmaeus*. J Mar Biol Ass UK 75:885-897

Shikata T, Shirata K (1999) Elevation of acid protease activity in the mantle muscle of female Japanese common squid *Todarodes pacificus* during maturation. Fish Sci 65:478-481

Skajaa K, Fernö A, Folkvord A (2004) Ontogenetic- and condition-related effects of starvation on responsiveness in herring larvae (*Clupea harengus* L.) during repeated attacks by a model predator. J Exp Mar Biol Ecol 312:253-269

Slip DJ (1995) The diet of southern elephant seals (*Mirounga leonina*) from Heard Island. Can J Zool 73:1519-1528

Smale MJ (1996) Cephalopods as prey. IV. Fishes. Phil Trans R Soc Lond B 351:1067-1081

Spranza JJ, Stanley EH (2000) Condition, growth, and reproductive styles of fishes exposed to different environmental regimes in a prairie drainage. Environ Biol Fish 59:99-109

Steer MA, Moltschaniwskyj NA, Nichols DS, Miller M (2004) The role of temperature and maternal ration in embryo survival: using the dumpling squid *Euprymna tasmanica* as a model. J Exp Mar Biol Ecol 307:73-89

Swift K, Johnston D, Moltschaniwskyj N (2005) The digestive gland of the southern dumpling squid (*Euprymna tasmanica*): structure and function. J Exp Mar Biol Ecol 315: 177-186

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fisheries Sci 11:107-184

Troedsson C, Grahl-Nielsen O, Thompson EC (2005) Variable fatty acid composition of the pelagic appendicularian *Oikopleura dioica* in response to dietary quality and quantity. Mar Ecol Prog Ser 289:165-176

Tveiten H, Jobling M, Andereassen I (2004) Influence of egg lipids and fatty acids on egg viability, and their utilization during embryonic development of spotted wolf-fish, *Anarhichas minor* Olafsen. Aquaculture Res 35:152-161

van Noordwijk AJ, de Jong G (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. Am Nat 128:137-142

Vidal EAG, DiMarco P, Wormuth JH, Lee PG (2002) Influence of temperature and food availability on survival, growth and yolk utilization in hatchling squid. Bull Mar Sci 71:915-931

Vidal EAG, Haimovici M (1998) Feeding and the possible role of the proboscis and mucus cover in the ingestion of microorganisms by rhynchoteuthion paralarvae (Cephalopoda: Ommastrephidae). Bull Mar Sci 63:305-316

Villanueva R (1992) Interannual growth differences in the oceanic squid *Todarodes angolensis* Adam in the northern Benguela upwelling system, based on statolith growth increment analysis. J Exp Mar Biol Ecol 159:157-177

Volkman JK, Nichols PD (1991) Applications of thin-layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine and environmental samples. J Planar Chromatography-Modern TLC 4:19-26

Wadley V, Dunning M (1998) Cephalopods of commercial importance in Australian fisheries. CSIRO Marine Research, Hobart

Waluda CM, Rodhouse PG, Podestra GP, Trathan PN, Pierce GJ (2001) Surface oceanography of the inferred hatching grounds of *Illex argentinus* (Cephalopoda: Ommastrephidae) and influences on recruitment variability. Mar Biol 139:671-679

Waluda CM, Trathan PN, Rodhouse PG (2004) Synchronicity in southern hemisphere squid stocks and the influence of the Southern Oscillation and Trans Polar Index. Fish Oceanogr 13:255-266

Watanabe K, Sakurai T, Segawa S, Okutani T (1996) Development of the ommastrephid squid *Todarodes pacificus*, from fertilized egg to rhynchoteuthion paralarva. Am Malac Bull 13: 73-88

Wiegand MD, Johnston TA, Martin J, Leggett WC (2004) Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Can J Aquat Sci 61:110-121

Wormuth JH (1998) Workshop deliberations on the Ommastrephidae: a brief history of their systematics and a review of the systematics, distribution, and biology of the genera *Martialia* Rochebrune and Mabille, 1889, *Todaropsis* Girard, 1890, *Dosidicus* Steenstrup, 1857, *Hyaloteuthis* Gray, 1849, and *Eucleoteuthis* Berry, 1916. Smith Cont Zool 586:373-383

Wormuth JH, O'Dor RK, Balch N, Dunning MC, Forch EC, Harman RF, Rowell TW (1992) Family Ommastrephidae Steenstrup, 1857. Smith Cont Zool 513:105-119

Yaragina NA, Marshall CT (2000) Trophic influences on interannual and seasonal variation in the liver condition index of northeast Arctic cod (*Gadus morhua*). ICES J Mar Sci 57:42-55

Yatsu A, Watanabe T, Mori J, Nagasawa K, Ishida Y, Meguro T, Kamei Y, Sakurai Y (2000) Interannual variability in stock abundance of the neon flying squid, *Ommastrephes bartramii*, in the North Pacific Ocean during 1979-1998: impact of driftnet fishing and oceanographic conditions. Fish Oceanogr 9:163-170

Young JW, Bradford RW, Lamb TD, Lyne VD (1996a) Biomass of zooplankton and micronekton in the southern bluefin tuna fishing grounds off eastern Tasmania, Australia. Mar Ecol Prog Ser 138:1-14

Young JW, Jordan AR, Bobbi C, Johannes RE, Haskard K, Pullen G (1993) Seasonal and interannual variability in krill (*Nyctiphanes australis*) stocks and their relationship to the fishery for jack mackerel (*Trachurus declivis*) off eastern Tasmania, Australia. Mar Biol 116:9-18

Young JW, Lamb TD, Bradford RW (1996b) Distribution and community structure of midwater fishes in relation to the subtropical convergence off eastern Tasmania, Australia. Mar Biol 126:571-584

APPENDIX 1.

Additiona biological information collected on female individuals of *Todarodes filippovae* during this study. Note that I did not collect the age data but is here presented to show how relationships change with growth of the squid. The age/growth data will be submitted as a separate study of which I am a collaborator.

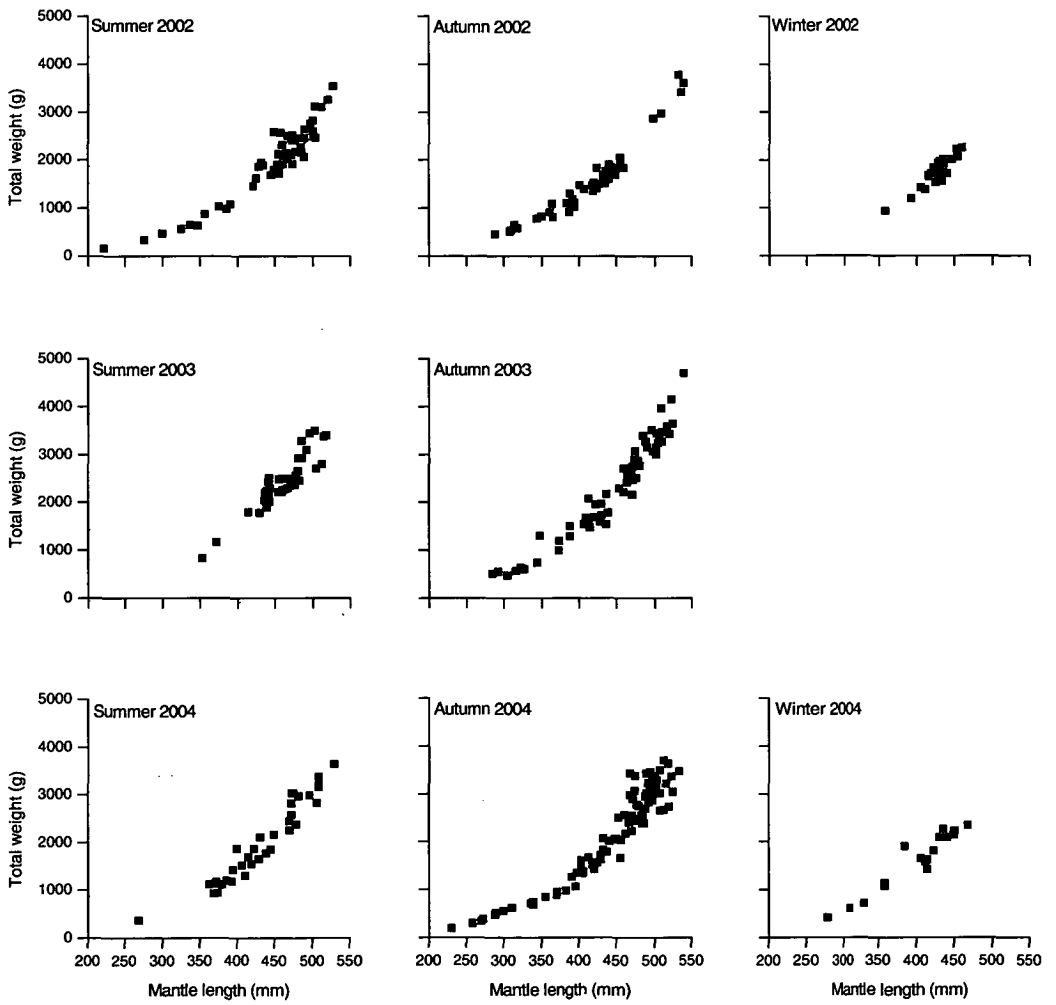

Figure 1a: *Todarodes filippovae*. The relationship between mantle length (mm) and total weight (g) for all female individuals for each season and year of capture.

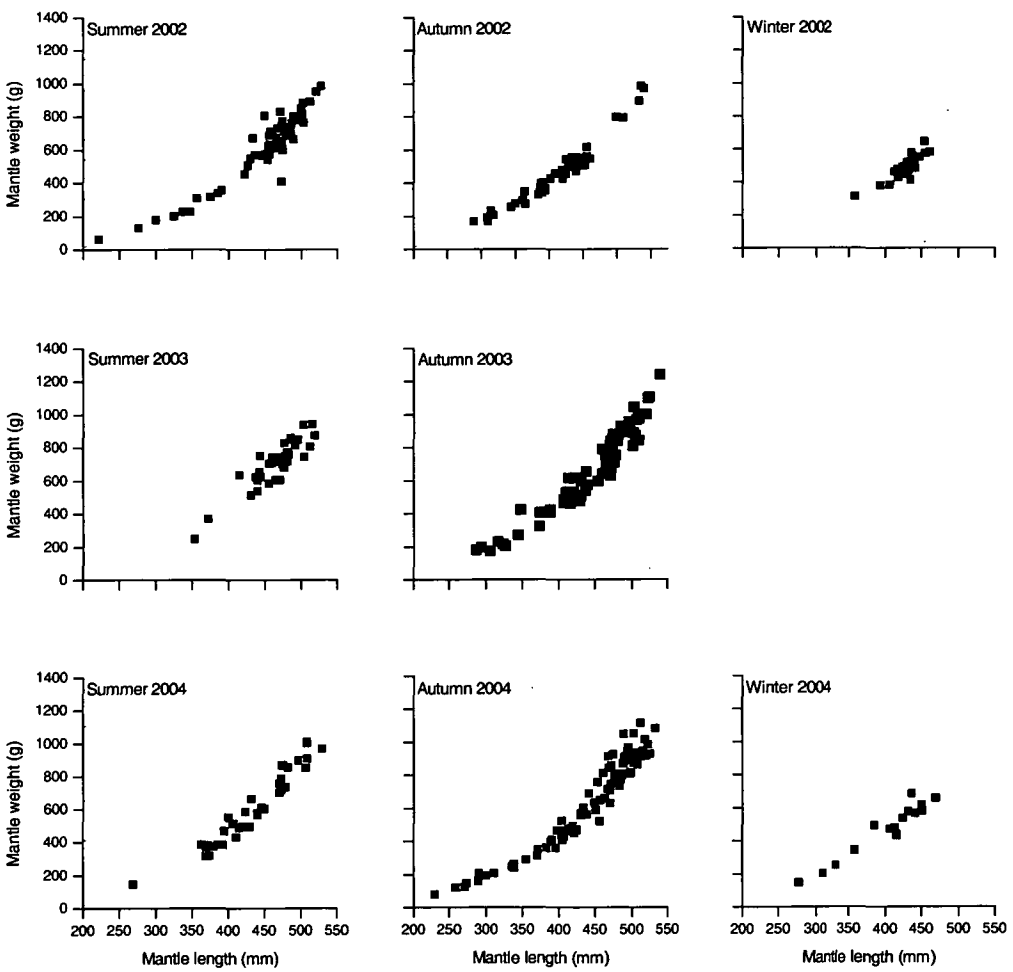

Figure 1b: *Todarodes filippovae*. The relationship between mantle length (mm) and mantle weight (g) for all female individuals for each season and year of capture.

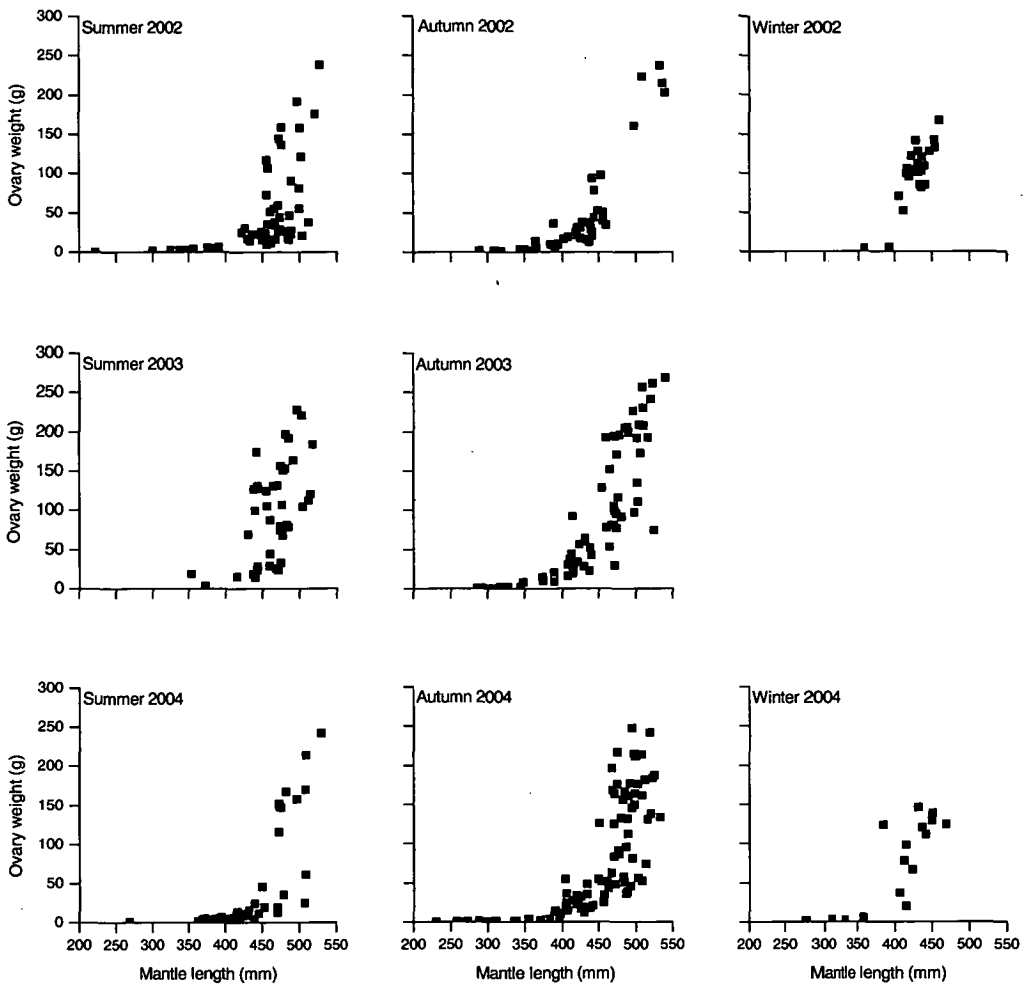

Figure 1c: *Todarodes filippovae*. The relationship between mantle length (mm) and ovary weight (g) for all female individuals for each season and year of capture.

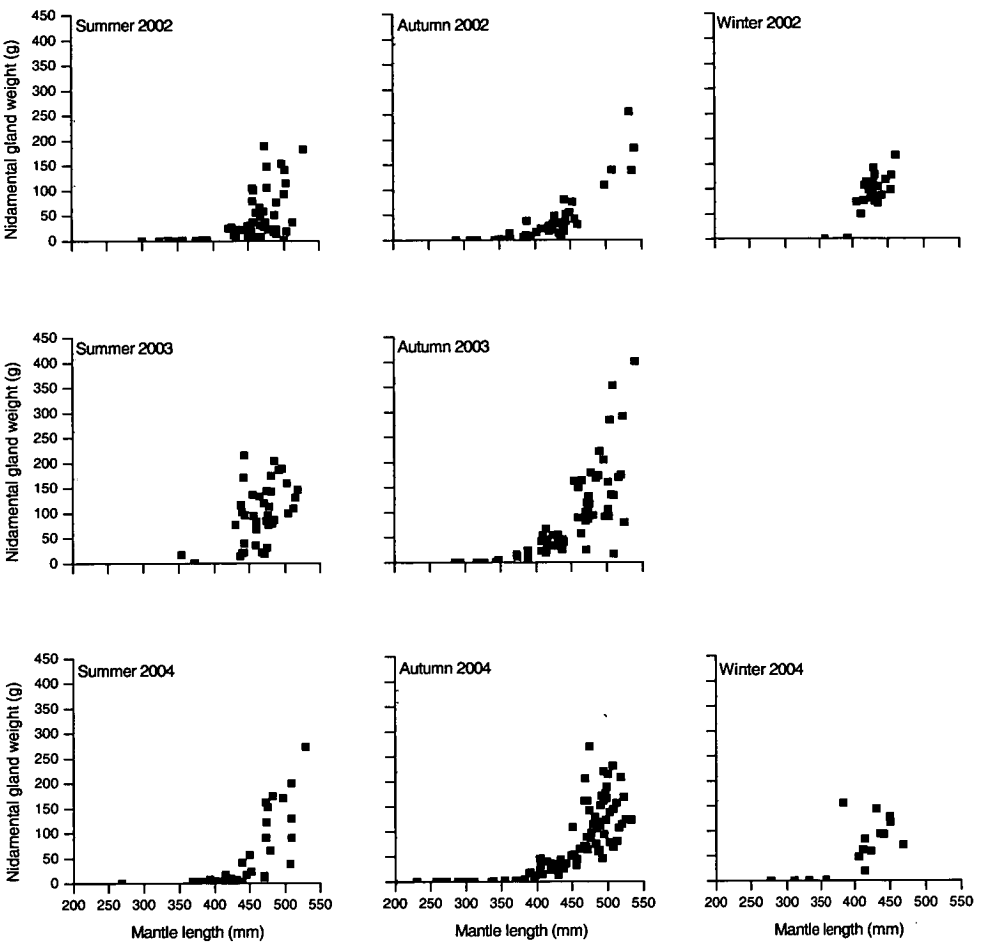

Figure 1d: *Todarodes filippovae*. The relationship between mantle length (mm) and nidamental gland weight (g) for all female individuals for each season and year of capture.

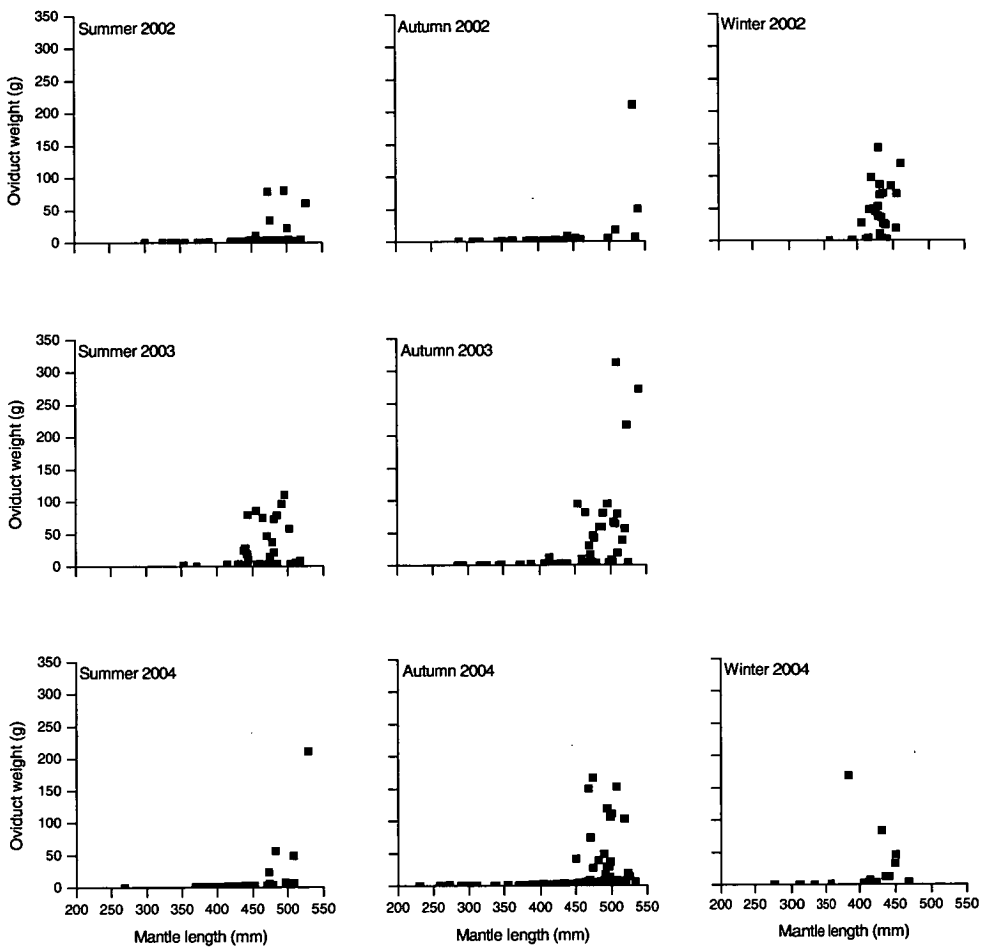

Figure 1e: *Todarodes filippovae*. The relationship between mantle length (mm) and oviduct weight (g) for all female individuals for each season and year of capture.

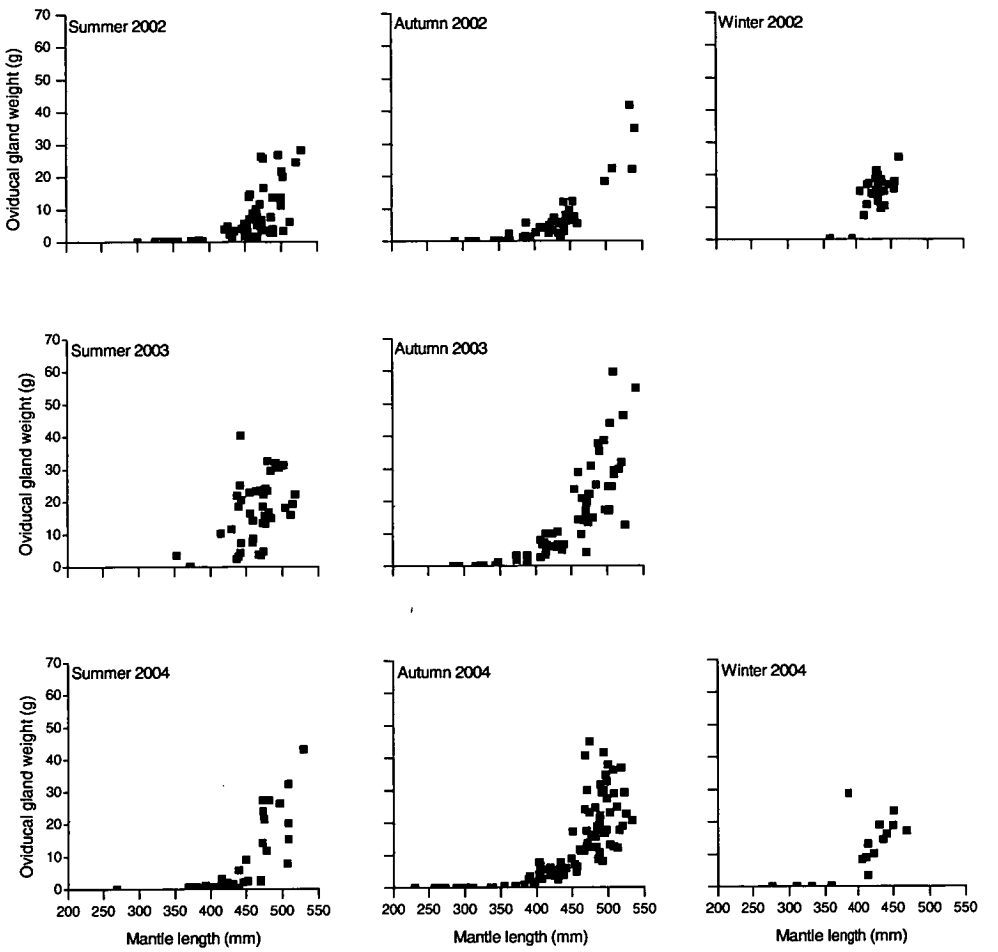

Figure 1f: *Todarodes filippovae*. The relationship between mantle length (mm) and oviducal gland weight (g) for all female individuals for each season and year of capture.

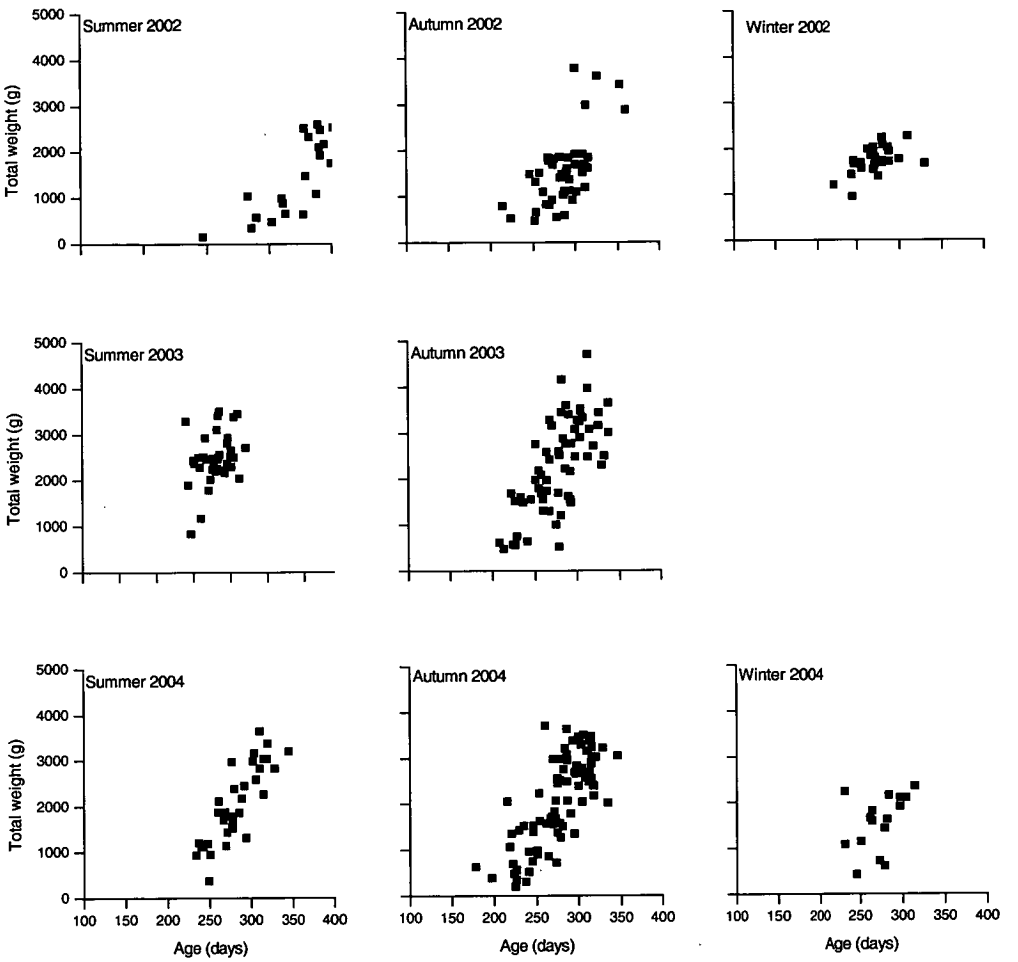

Figure 1g: *Todarodes filippovae*. The relationship between age (days) and total weight (g) for all female individuals for each season and year of capture.

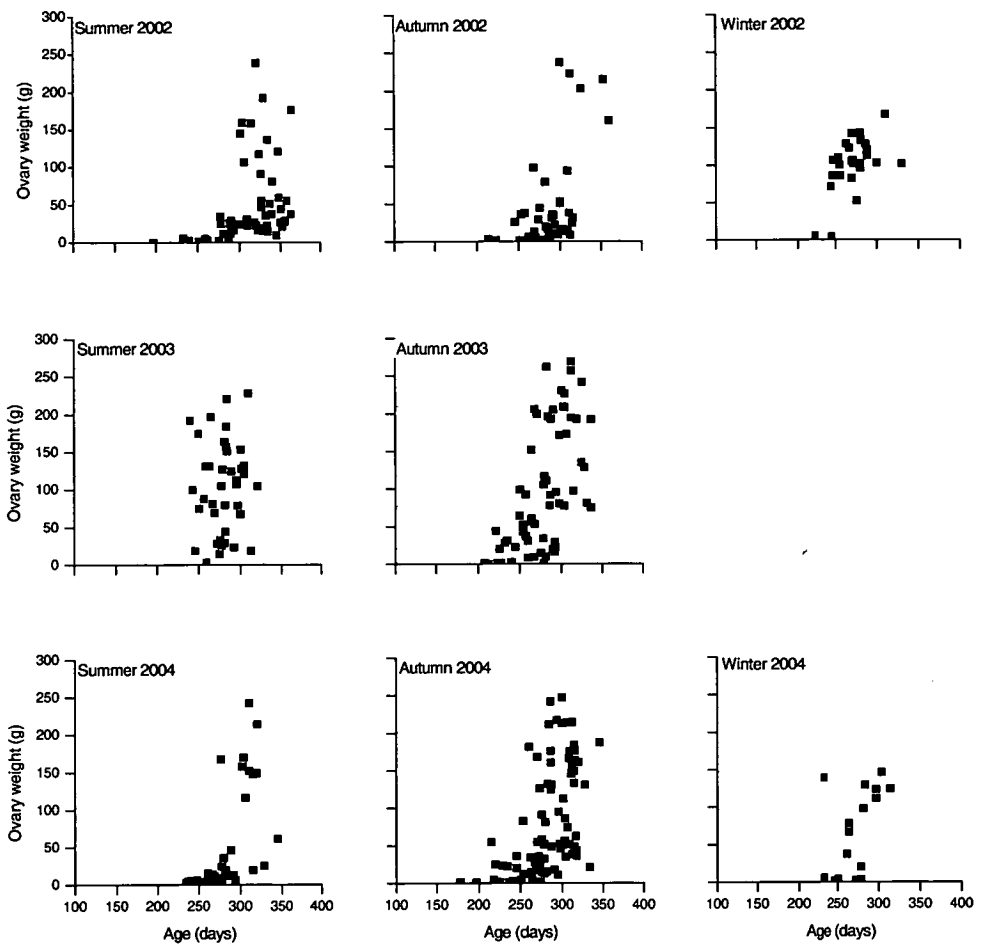

Figure 1h: *Todarodes filippovae*. The relationship between age (days) and ovary weight (g) for all female individuals for each season and year of capture.

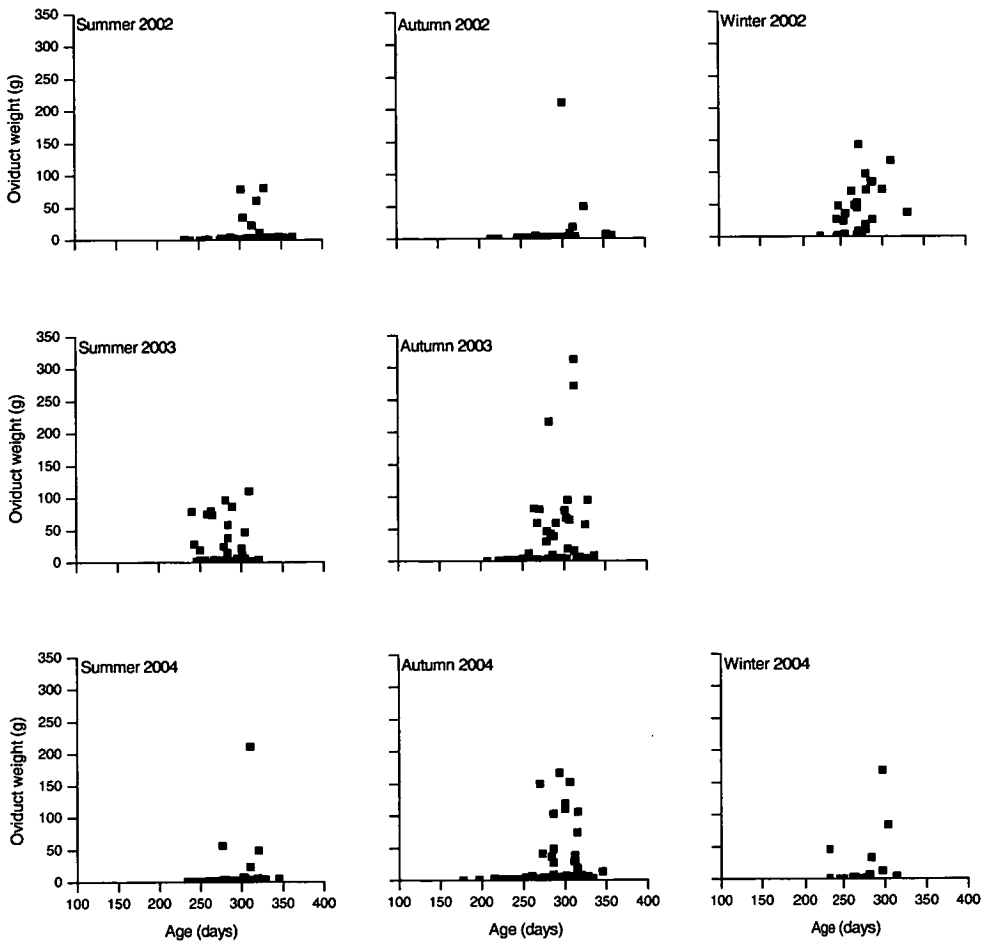

Figure 1i: *Todarodes filippovae*. The relationship between age (days) and oviduct weight (g) for all female individuals for each season and year of capture.

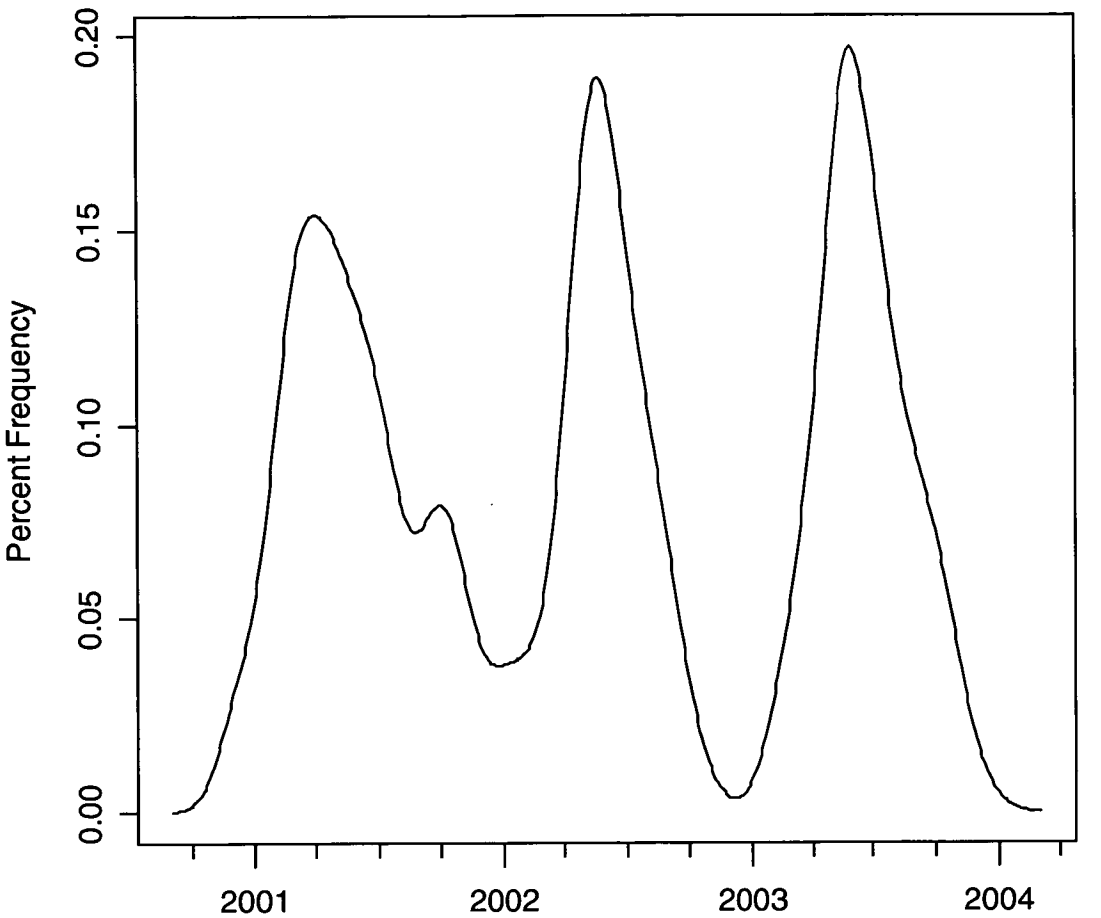

Figure 1j: *Todarodes filippovae.* Hatch date distribution for all aged individuals captured over the study period.